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# THE JOURNAL

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# EXPERIMENTAL ZOÖLOGY

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## CORRIGENDA.

- p. 200, l. 4: for "vegetable" read "vegetative."  
p. 201, l. 27: after "both" insert "somatoblasts."  
l. 30: omit "CD."  
p. 209, l. 9: before "prototrochal" insert "the."  
p. 210, l. 7: for "circle" read "row."  
p. 212, l. 4: for "size-relation" read "size-relations."  
p. 217, l. 7: for "ciliation" read "division."  
p. 236, l. 14: for "are" read "is."  
p. 237, l. 13: insert period after "forms."  
l. 34: for "disintegrated" read "disintegrate."  
p. 244, l. 30: for "essentially" read "in many of its features."  
p. 247, l. 12: omit "the importance of."  
l. 13: omit "of."  
l. 14: for "has" read "have."  
p. 248, l. 16: for "casual" read "causal."  
p. 253, l. 33: insert hyphen after "entoblast."  
p. 260, l. 8: for "Ganzbeziehungsweise" read "Ganz- beziehungsweise."  
p. 267, l. 1: for "Studien" read "Stadien."





# EXPERIMENTAL STUDIES ON GERMINAL LOCALIZATION.

BY  
EDMUND B. WILSON.

## I. THE GERM-REGIONS IN THE EGG OF DENTALIUM<sup>1</sup>.

WITH 100 FIGURES.

### CONTENTS.

- I. Introduction.
- II. Preliminary Observations on the unsegmented Egg and the normal Development.
- III. Effect of removing the Polar Lobe.
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- IV. Localization of the apical Organ, and its Correlation with the post-trochal Region.
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- VII. Comment.
- VIII. Summary.

### INTRODUCTION.

The following experimental studies are offered as a contribution to the theory of "Organbildende Keimbezirke" or germinal prelocalization, especially as applied to the cytoplasmic regions of the unsegmented egg. Following the enunciation of the principle of "precocious segregation" by Ray Lankester, in 1877, the im-

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<sup>1</sup>This work was carried out at the Naples Zoological Station between February and August, 1903, on a grant from the Carnegie Institution of Washington, in which was included the use of one of the tables subscribed for by the Institution. My best thanks are due to the administration of the station for the unfailing efficiency and courtesy with which my work was aided in every possible way.

portance of the cytoplasmic factors of localization and differentiation was early recognized by Whitman in his remarkable paper on *Clepsine* ('78) and emphasized by him in later papers. Similar views were more or less clearly expressed by Van Beneden, Flemming, Platner and others prior to the definite formulation of the mosaic-theory of development by Roux in 1888.<sup>1</sup> Roux himself recognized from the first, as a prominent factor in his theory, the importance of a definite topographical grouping of specific cytoplasmic materials in the unsegmented egg; though unfortunately this was complicated, then and in later discussions, by the hypothesis of qualitative nuclear division, which has since been shown to be untenable and has now been relinquished by its author (Roux, 1903). Since that time the evidence, both cytological and experimental, has steadily increased that a prelocalization of the morphogenic factors in the cytoplasmic regions is a leading factor in the early development; and it has become evident that this is true not only in such "mosaic eggs" as those of mollusks or ctenophores, but even in those of echinoderms or nemertines, where an isolated blastomere or an egg-fragment may produce a perfect dwarf embryo. It has become of high importance to determine experimentally in what degree such prelocalization or cytoplasmic "organization" may exist in the unsegmented egg, and to what extent it may vary in different forms. It is even more important for our general conception of development to determine by the same method whether the prelocalization of the morphogenic factors, in whatever degree it may occur, exists from the beginning, or whether, as the cytological evidence seems to show, it is established by a progressive process; for in the latter case, as is hardly necessary to point out, prelocalization, even in the unsegmented egg, may be brought under the category of epigenetic phenomena ("epigenetic qualities" as distinguished from "preformed qualities"<sup>2</sup>), and falls into harmony with hypotheses that assume the nucleus to be the primary determining factor.

The present studies, which are a continuation of the preceding

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<sup>1</sup> Cf. my work on *The Cell*.

<sup>2</sup> Roveri ('03), p. 356.



ones on the nemertine egg (Wilson, '03) bear upon both these questions. In that paper I approached especially the second question in an experimental study of the egg of *Cerebratulus*, which has since been extended by the work of Yatsu ('04). My results clearly showed that in this egg the cleavage-factors are not definitely localized until after the completion of the maturation of the egg, but they gave no definite evidence regarding the localization of the morphogenic factors (as distinguished from those of cleavage) at this period; it was, however, shown that in the comparatively young blastula, before the formation of the mesoblast, morphogenic localization, as shown in the pre-determination of the gut and apical organ, has become much more definite than in the unsegmented egg. Yatsu subsequently obtained evidence, in the same species, that the localization of the morphogenic factors is a progressive process even in the stages preceding cleavage, since the percentage of normal larvae obtained from egg-fragments at successive periods steadily diminishes from the first discharge of the eggs (when maturation begins) up to the period immediately preceding the first cleavage; and the nature of the defective larvae, correlated with the plane of section, pointed to a increasingly definite localization, in the later stages preceding cleavage, of the bases of several important organs, such as the apical organ, gut, and ciliated lobes of the pilidium. I am now able to offer an experimental analysis along the same lines—perhaps I should say the beginning of such an analysis—of the molluscan egg, in which pure observation of the cell-lineage has produced such convincing evidence of mosaic development, sustained by Crampton's initial experimental examination of the gasteropod egg ('96), and by the interesting cytological work of Lillie ('01) and Conklin ('02) on the cytoplasmic regions of the unsegmented and segmenting egg. The cytological and experimental results coincide in demonstrating in this egg (specifically in *Dentalium*) the existence of a very definite prelocalization of some of the most important factors both of cleavage and morphogenesis, which here closely coincide. They show conclusively also, contrary to what the nemertine experiments had led me to expect, that in its main features this

prelocalization exists in the egg at the time it leaves the ovary, and probably much earlier, and long before even the initial stages of maturation and fertilization. Nevertheless, progressive changes take place during and subsequent to maturation, which, when compared with those occurring in other forms, show this egg, as I believe, to be only the extreme of a series that connects it with such forms as the nemertine or echinoderm, and brings them under one point of view.

The present paper deals mainly with the development of fragments of the unfertilized egg of *Dentalium*, the eggs being cut singly with the scalpel under the microscope and subsequently fertilized, following the method of Delage ('99). I shall here consider the development of isolated blastomeres only incidentally for the sake of comparison, reserving a fuller account for a second paper. It may be stated here, however, that the experiments on this part of the subject demonstrate, even more conclusively than do those of Fischel for the ctenophore-egg, that the cleavage of the ovum, in both *Dentalium* and *Patella*, is in fact what the normal cell-lineage so clearly indicates, essentially a mosaic-work, in accordance with Crampton's earlier experiments on *Ilyanassa*. Blastomeres isolated at any stage from the 2-cell onward continue to segment as if still forming part of a complete embryo; and apart from the changes due to shifting of the cells, which, as in the ctenophore, often lead to the displacements of the larval structures and to the closing of the partial embryos, undergo essentially the same differentiation as if united to their fellows. Thus, the first two blastomeres, upon separation, give rise to two dissimilar larvae, each of which is defective and represents essentially the same structures as would have been produced had the two cells remained united; in like manner, of the isolated cells of the 4-cell stage, the larva from the D-quadrant possesses certain structures that are lacking in the other three; and the differences among the larvae from cells of the 8- or 16-cell stages are still greater. Cells procured by successive isolations up to the 64-cell stage, or later, differentiate singly, according to their nature, into actively swimming trochoblasts of three kinds; into ordinary ectoblast- or entoblast-cells, into sensory cells bearing

the characteristic sensory hairs of the apical organ; and even into what I believe to be muscle-cells and mesenchyme-cells, though, unlike the foregoing cases, the precise origin of these was not traced. These eggs thus represent the opposite extreme to such forms as those of *Amphioxus*, the echinoderm, or the nemertine, and give a result which, apart from the hypothesis of qualitative nuclear division, agrees essentially with Roux's original conception of mosaic-development, with the conclusions of many students of cell-lineage, with the experimental results of Crampton on the gasteropod-egg, and with those of Fischel regarding the ctenophore-egg.<sup>1</sup>

## II.

### PRELIMINARY OBSERVATIONS ON THE UNSEGMENTED EGG AND THE NORMAL DEVELOPMENT.

The egg of *Dentalium*, like that of *Cerebratulus*, possesses certain features by means of which the axis may be determined in the living egg from the moment of its release from the ovary. The egg is more or less deeply pigmented, perfectly opaque, and of a color that varies in different individuals from light olivaceous to reddish brown or almost brick red. When first set free the egg is somewhat irregular, but quickly becomes more rounded. It is then seen to be very considerably flattened, so as often to be almost biscuit-shaped, one side being always more flattened than the other, and often more or less irregular in contour. Viewed by reflected light the central region of each of the flattened sides is seen to be occupied by a very distinct, though vaguely bounded, white area, nearly or quite free from pigment (Fig. 1); these areas, as shown by the subsequent development, correspond with the two poles of the egg, and the more flattened side, which

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<sup>1</sup> The eggs of *Patella*, which were employed mainly for a study of the isolated blastomeres, were available from the middle of March until the latter part of May. Those of *Dentalium*, which were used especially for the development of egg-fragments, first became mature at the beginning of June, when less than two months remained for their study. The shortness of this period accounts for some of the obvious gaps in my work. The complexity of the subject, and the practical difficulties presented by the material are such that more extended work, with additional material, will be required for its completion.



is the side of attachment in the ovary, is found to represent the lower or vegetative hemisphere.

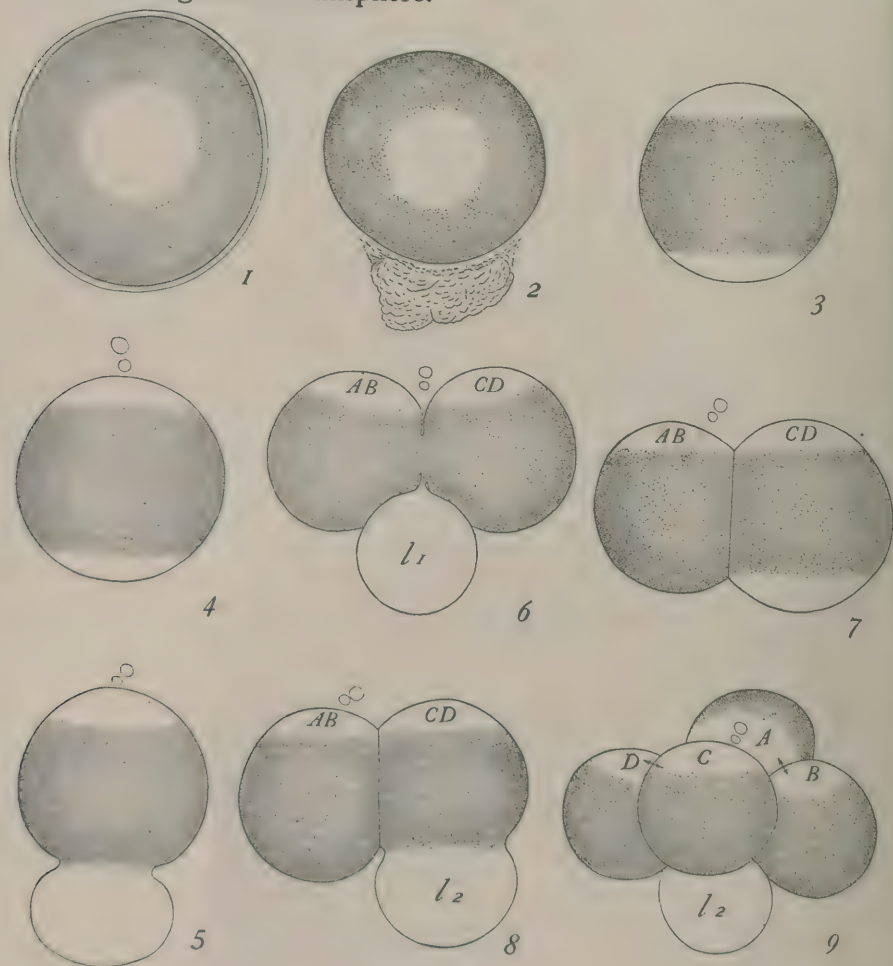


FIG. 1.

*Cleavage, from living Eggs.*

1, Outline of egg soon after release, in polar view, showing white polar area; 2, the same egg, 20 minutes later, after throwing off the membrane; 3, similar egg, from the side; 4, egg one hour after fertilization, with fertilization-membrane<sup>1</sup> and polar bodies; 5, beginning of the first cleavage, formation of the polar lobe; 6, trefoil, 1¼ hours after fertilization; 7, resulting 2-cell stage; 8, beginning of second cleavage from the side, second polar lobe forming; 9, second cleavage at its height.

<sup>1</sup> Accidentally omitted by engraver.

During the 20-30 minutes following its release the ripe, unfertilized egg becomes nearly spherical (and hence appears considerably smaller in polar view), the membrane by which it is at first surrounded separates more widely from the egg, finally ruptures suddenly, and then quickly draws together at one side, where it is thrown off as a mass of *débris* attached to the egg (Fig. 2).<sup>1</sup> Following this, a substance which at first surrounds the egg as a thin, transparent layer swells up to form a jelly, which raises the egg slightly from the bottom. The wall of the germinal vesicle breaks down at about this period (20-30 m.), leaving a clearer space in which the first maturation-figure appears. The white polar areas are still clearly visible, and the egg, still unfertilized, now gives the appearance of being surrounded by a very broad, horizontal pigment-ring, which, though often faint and with vague boundary, is always distinctly visible (Figs. 3, 4). The ring recalls that described by Boveri ('01) in the egg of *Strongylocentrotus*, though relatively broader. The egg of *Dentalium* thus shows a visible stratification of material analogous to the zones seen in *Strongylocentrotus*; but, unlike the latter, the zones of *Dentalium* clearly pre-exist before even the preparatory changes of maturation take place.

Sections and total preparations of the flattened egg, fixed shortly after its discharge or removal from the ovary, show that a distinct structural modification exists in each of the white areas, at this period much more marked in case of the lower or vegetative area. Surrounding the lower pole (Fig. 10) is a very distinct mass of dense almost homogeneous protoplasm, of approximately the same

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<sup>1</sup> All the figures were outlined as accurately as possible with the camera, and with the exception of Figs. 10-13 and 33, 38-41, are enlarged to the same scale (150 diameters). They are only schematized in that the pigment is represented by stippling, whereas the color does not actually appear in the form of distinct granules, but as a nearly uniform hue. The stippling somewhat exaggerates the distinctness of the pigment as seen in most individuals; though in the most deeply pigmented ones, viewed under strong direct light, the color appears with great distinctness and its limits may be clearly seen. The operation of cutting usually leads to disturbances in the arrangement of the pigment, so that frequently no definite color-pattern can be clearly made out in the dwarf embryos. I have only represented the pigment in cases where its boundaries could actually be seen.

extent as the white area seen in the living egg; this contains no yolk-spheres, and stains with great intensity with a strong plasma-stain like Congo red. This mass, sharply marked off from the surrounding yolk, bulges slightly outward at the surface and at the margin is continuous with a very thin ectoplasmic zone that entirely surrounds the egg, but is only clearly visible in sections. Internally this mass is confluent with a somewhat narrow zone of similar finely granular protoplasm that extends upwards partly around the germinal vesicle. It is probably to the presence of this remarkable protoplasmic mass that the appearance of the lower white area is due, though the latter may have a different cause. In a general way, the lower protoplasmic area is undoubtedly comparable with the lower zone, composed of green material, seen in the egg of *Myzostoma* (Beard, Wheeler, and Driesch), as is proved by its later history. Comparison of my Fig. 10 with Wheeler's Fig. 2 ('97), will show how closely similar the relations of the lower protoplasmic area in the two eggs are.<sup>1</sup>

The upper white area cannot be distinguished as such in the fixed eggs, and is apparently produced by a different cause from the lower one. Exactly at the upper pole is a very small, superficial disc of clear, dense, intensely staining protoplasm, which, like the lower protoplasmic mass, is continuous at its margin with the general ectoplasmic layer (Fig. 10). This upper disc is so small as readily to escape observation; but sufficiently careful examination invariably reveals its presence, which is furthermore frequently indicated by a slight indentation of the egg-periphery at this point. It varies considerably in thickness and extent in different specimens, but is always very small at the beginning.<sup>2</sup> Evidently, the upper protoplasmic disc is not large enough to account for the appearance of the upper white area in the living egg, which must be due to some other cause,

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<sup>1</sup> Compare also Driesch, '96, Fig. 12.

<sup>2</sup> Sections of the ovary show that both the upper disc and the lower protoplasmic area are present while the egg is still attached to the ovarian wall. The eggs are greatly distorted in shape, but in a general way are pyriform, and attached by the narrow end. The lower protoplasmic area occupies the narrower end, by which the eggs are attached; the upper disc is at the opposite point.



perhaps to a lighter tint in the deutoplasm in this region. In the following account, accordingly, it will be necessary always to distinguish clearly between the upper white area, or polar area, and the upper protoplasmic disc or area.

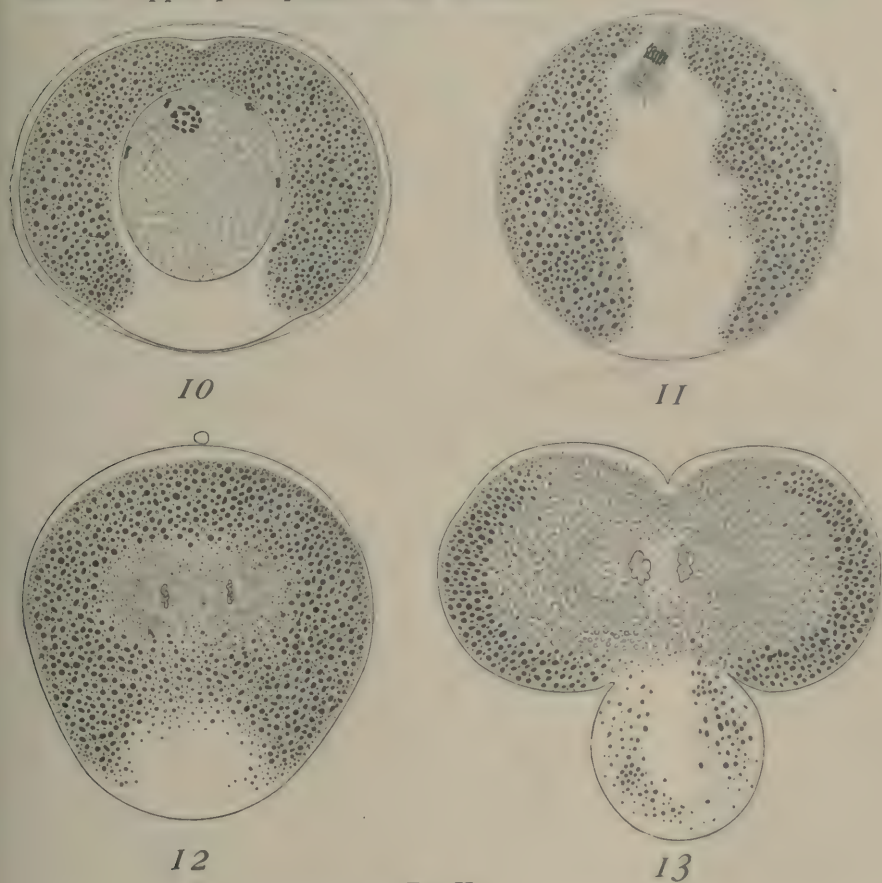


FIG. II.

*Vertical Sections of the Normal Egg.*

Fig. 13 directly from section (picro-acetic); outlines of Figs. 10-12 (sublimate-acetic) from optical section of total preparations, details from actual sections. The peripheral zone of deeply staining yolk shown in Fig. 13 occurs in all these stages after picro-acetic fixation, but not after sublimate-acetic.

10, Unfertilized egg, five minutes after release, showing both protoplasmic areas; chromosome-like bodies in the nucleolus; 11, fertilized egg, 30 minutes after fertilization, first polar spindle; 12, fertilized egg, 60 minutes after fertilization, initial stage in formation of polar lobe; 13, first cleavage, 68 minutes after fertilization, just before the complete trefoil stage.

I shall here give only a very general account of the later history of the two protoplasmic areas, which will require a thorough cytological study for its full elucidation. As the egg, still unfertilized, lies in sea-water, the ectoplasm in the region of the upper disc slowly increases in amount, and in some cases this region shows a faintly radiating appearance around its periphery as if clear hyaloplasm were flowing into it from the surrounding region. I am uncertain whether in this process the original disc itself enlarges or is only surrounded by an accumulation of hyaloplasm — a point of importance for the comparison with the upper polar ring of the annelid egg that is drawn further on. I shall continue to speak of the ectoplasmic thickening at the top of the egg as the “upper protoplasmic area,” but would call attention especially to the fact that the original disc is composed of very dense homogeneous protoplasm that differs markedly in character from the alveolar protoplasm of the ectoplasmic thickening that afterwards extends over the whole upper surface of the egg.<sup>1</sup>

When the germinal vesicle breaks down, the maturation-spindle, which is relatively small, is formed just below this protoplasmic area, rotating into a radial position and moving towards the periphery so that its outer end lies in or just below it (Fig. 11). In this position it remains, in metaphase, until the egg is fertilized, when the divisions proceed, the polar bodies being successively extruded exactly at the upper pole, at the centre of the upper protoplasmic area (which is now rapidly extending and shows no definite boundary), and hence at the centre of the upper white area (Fig. 4). At this period the protoplasmic area comes into connection by a rather narrow neck of hyaloplasm, in which the spindle lies, with the central mass left after the germinal vesicle breaks down. After the polar bodies are formed this connection is severed, and the upper protoplasmic area spreads out still more

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<sup>1</sup> The general ectoplasmic layer can in the earlier stages hardly be seen in total preparations, but appears clearly in sections either after staining with haematoxylin and a strong plasma-stain such as Congo red (when it appears clear red) or after borax carmine. It is at first much thinner and less definitely bounded than, for instance, in *Rhynchelmis* as figured by Vejdovsky, '88 (in the recent paper of Vejdovsky and Mrazek, '03, it is represented as much thinner than in the earlier paper), but later becomes very conspicuous.

widely so as to appear as a general thickening of the ectoplasmic layer over the whole upper hemisphere (Figs. 12, 13). This thickening is most marked near the animal pole, where it is very conspicuous at the time of cleavage, extending thence approximately to the equator of the egg, or slightly below it, but without any very definite margin. It stains deep red in Congo red and shows a finely alveolar structure quite unlike that of the original disc.

During the foregoing stages marked changes occur also in the lower protoplasmic area, and it is evident that active movements of its material take place. These are perhaps due in part to the entrance of the spermatozoon at the lower pole, but in part also to the fact that upon the breaking down of the germinal vesicle the finely granular material derived from it becomes more or less definitely confluent with the lower area (as Wheeler describes in *Myzostoma*), so that an irregular pillar of protoplasm, surrounded on all sides by yolk, now extends from the lower pole nearly to the upper protoplasmic area (Fig. 11) and ultimately becomes connected with the latter as the first maturation spindle moves upwards.<sup>1</sup> In vertical section it may very clearly be seen that the material of the upper part of this pillar differs markedly from the lower, both in texture and in staining capacity (the two regions show a rather distinct boundary, indicated by the dotted line in Fig. 11), the lower region being very dense and staining in the double stain clear red, the upper one much looser (alveolar?) in structure and staining purple or blue. During the polar body formation the lower area changes its form, often becoming irregular and sometimes elongate or sickle-shaped. It is a noteworthy fact that at the time each polar body is extruded the egg becomes irregular in contour or almost amoeboid, at the center of the lower polar area, afterwards resuming its even outline.<sup>2</sup> After formation of the polar bodies the upper part of the protoplasmic pillar retreats from the periphery, while the yolk again extends across the upper region above the egg-nucleus. In the upper part of the internal protoplasmic region conjugation of the

<sup>1</sup> Cf. Wheeler's Fig. 10 or 16.

<sup>2</sup> This was figured by Lacaze Duthiers ('57) nearly fifty years ago.



germ-nuclei takes place. At the period shortly preceding the first cleavage, when the upper disc has been replaced by the very broad ectoplasmic thickening described above, the lower protoplasmic area, as seen in surface views of total preparations, varies a good deal in appearance in different individuals, being sometimes rounded and fairly well circumscribed, sometimes irregular, or even broken up so as to present a mottled appearance.

The first cleavage, which occurs about thirty minutes after the extrusion of the second polar body, is characterized by a trefoil stage, like that occurring in many gasteropods, lamellibranchs and annelids (Figs. 5, 6). Exactly surrounding the lower pole is formed, by a horizontal constriction, a large lobe, into which passes the whole of the lower white polar area, and which, like the area itself, appears pure white in the living object. Since the surface of the lobe is much larger than that of the original lower polar area from which it arises, it is evident that material from the interior of the egg must flow into the lobe as it forms. Vertical sections of the egg as the polar lobe begins to form show somewhat varying appearances, due in part to differences in the plane of section, but also in part to varying conditions in the protoplasmic area itself. The rather small cleavage-figure, at this period entirely surrounded by deutoplasm, lies in late anaphase or early telophase slightly above the centre of the egg. At the lower pole the dense protoplasm of the lower area is now spread out, more or less irregularly, to form a thick peripheral layer that fades away insensibly into the yolk-bearing region. Frequently, as in Fig. 12 (*cf.* Wheeler's Fig. 46) this thickening appears fairly regular and symmetrical and suggests the ectoplasmic thickening that precedes the formation of a pseudopod in *Amæba*; sometimes it is less regular than this, and occasionally gives the appearance of an asymmetrical wedge-shaped mass extending into the yolk. As the lobe forms it receives this clear protoplasm, accompanied by an inflow of yolk that seems to invade the clear substance more or less; so that in section scattered yolk-granules are found in the lobe and frequently no definite boundary of the clear substance can be distinguished (Fig. 13). In any case it is certain that the whole of the lower protoplasmic

area passes into the lobe (like the green material of the *Myzostoma* egg) to constitute its main bulk, precisely as Wheeler shows in *Myzostoma* (cf. his Fig 47). The term "yolk-lobe" employed by a number of earlier observers is therefore as misleading as it is inappropriate and may be replaced by the term "polar lobe." For reasons given in the discussion at the end, I believe it very probable that at least the lower protoplasmic area, and probably also the upper disc, are in a general way comparable to, if not identical with, the polar rings observed in the eggs of certain leeches and oligochaetes.

Immediately after the polar lobe is formed a vertical furrow cuts into the egg from the upper pole, dividing the upper white area into equal parts and forming with the polar lobe a trefoil, of which the two upper lobes are of exactly equal size and contain all of the pigment, while the unpigmented polar lobe is considerably less than half the bulk of each of the others (measurements give a ratio of 1 to 0.32-0.46, Fig. 6). At the height of its formation the trefoil appears at first sight to consist of three separate spheres. Close examination invariably shows however that the polar lobe is united to one of the upper lobes by a very narrow pedicle which is never severed; and as the cleavage proceeds these two lobes completely fuse while the remaining upper lobe is cut off as a separate blastomere. Thus is formed a characteristic unequal 2-cell stage (Fig. 7), consisting of a smaller anterior cell, AB, and a larger posterior one, CD, which differ in volume by exactly the bulk of the polar lobe. Each of these cells has at the upper pole a white area, representing half the original upper polar area. The lower polar area, on the other hand, is confined to the larger cell, and obviously represents that part of the substance of the fused polar lobe that appears at the surface, a part having again moved into the interior of the egg.<sup>1</sup> Upon the 2-cell stage thus formed is moulded the entire subsequent development, which in its general outline is of essentially the same type as in such forms as *Unio* or *Nereis*.

The experiments recorded in this paper relate mainly to the significance of the material of the lower polar area, and of the polar lobe, and form a continuation of those begun by Crampton

<sup>1</sup> Cf. Wheeler's Fig. 48.

in his interesting experimental paper on *Ilyanassa*, published in 1896. In order to understand the significance of the experiments to be described it will be necessary to trace briefly the subsequent development. The second cleavage is ushered in by the reappearance of the polar lobe at the vegetative pole of the larger cell, CD, of the same size and form as before, and again consisting entirely of white material (Figs. 8, 9). The cleavage in this cell, whether separated from its fellow or remaining united

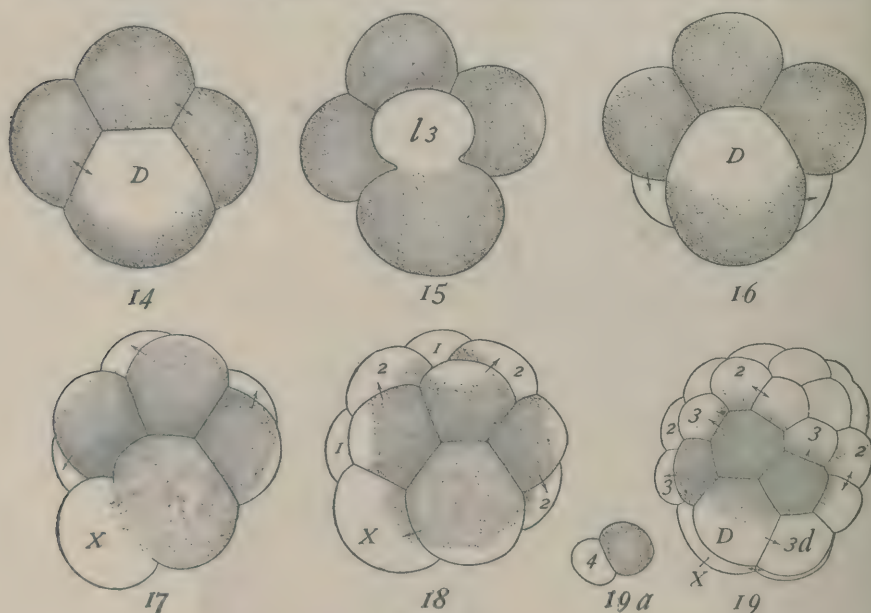


FIG. III.

*Cleavage, from living Eggs.*

14, Four-cell stage, from lower pole; 15, beginning of third cleavage, from lower pole, third polar lobe; 16, eight-cell stage, from lower pole; 17, beginning of fourth cleavage, first somatoblast in formation; 18, sixteen-cell stage, from lower pole; 19, view from lower pole, after the formation of the third quartet; 19a, D (pigmented) and 4d, immediately after division; surface view.

with it, follows the same general course as in the first cleavage of the entire egg, the polar lobe finally fusing with one of the cells, namely, D, the left posterior quadrant, where it again forms a very definite lower polar white area.<sup>1</sup> The anterior cell, AB,

<sup>1</sup> Cf. Wheeler's Fig. 49, Driesch's ('96) Fig. 12, of *Myzostoma*.



in the meantime divides equally, without the formation of a polar lobe. In the 4-cell stage, accordingly, the large posterior cell, D, exceeds A, B or C, by exactly the volume of the lobe, and the lower white area appears only in D (Fig. 14). On the other hand, the substance of the original upper white area is equally distributed among the four; but it is evident that the amount of white material visible at the surface has somewhat increased. The 4-cell stage shows the characteristic relations of the blastomeres observed in so many other eggs of this type. The two lateral cells, A and C, lie at a higher plane, and are in contact along the upper side by an upper "cross-furrow." B and D, on the other hand, are in contact along a longer transverse lower cross-furrow; and these characters, together with the large size of the posterior cell, D, thus give an immediate means of orientation from this time forwards.

As the egg prepares for the third cleavage the upper white material shifts slightly towards the left upper angle in each quadrant, anticipating the formation of the first quartet of ectomeres by the usual dextrotropic cleavage. These cells, which are of equal size and in the A, B and C quadrants are not much smaller than the basals, are formed entirely from the white material of the upper polar areas; and it is here again evident that an extensive flow of this material must take place from the interior of the egg. Their formation does not, however, exhaust the white substance of the upper areas, which still remain in the upper regions of the four basals. During this division the polar lobe forms for the third and last time, from the white material of the lower area, in the D-quadrant; but it is now noticeably smaller than before, and does not constrict so deeply (Fig. 15). After the completion of the cleavage the lobe again fuses with D, in which, as the egg enters into the "resting stage," the lower white area still appears; though this soon undergoes a great change (Fig. 16).

The fourth cleavage is of especial interest, since *a large part of the substance of the lower white area now passes into the first somatoblast, 2d, or X*, and is thus for the first time actually cut off from the pigmented region. This cleavage is preceded and

accompanied by an extensive shifting of the cytoplasmic materials in all of the cells. In the three basals, A, B and C, the white material towards the animal pole moves over towards the upper right angle of the cell and increases in amount, extending so far down the egg that in some individuals it may be seen, when the egg is viewed from the vegetative pole, as a narrow white crescentic area (Fig. 16). A similar process takes place in D, but in addition to this a great change takes place in the white material of the lower polar area, which leaves its position at the lower pole, moves over towards the same side as the upper white area, and finally fuses with it, while the pigmented part becomes lighter in color, often irregular or mottled in appearance, and extends into the area formerly occupied by the lower white substance. In the ensuing cleavage, D is usually the first to divide, giving rise by a leiotropic cleavage to the large first somatoblast, 2d or X (Figs. 17, 18). This cell consists almost entirely of white material which is certainly derived in large part from the original lower white area, but undoubtedly also in part from the upper white area, which, as stated above, fuses with the lower area in the period preceding this cleavage. In some cases X receives also a small amount of the pigment (Fig. 18), in others it seems to be composed entirely of white material. The other members of the second quartet, 2a, 2b, and 2c, are much smaller than X, and each is formed mainly from the white material of the upper polar area, but as a rule, perhaps always, each receives also a variable amount of pigment. During the foregoing changes the upper quartet divide leiotropically in the usual fashion, to form the four primary trochoblasts, which are slightly smaller than the upper cells. Owing to the foregoing changes the pigment, which in the unsegmented egg extended far up towards the animal pole, has been moved downwards so as to lie below the equator of the egg, most of it being contained in A, B and C, some in D, a little in 2a, 2b and 2c, and sometimes also a little in 2d. The pigment becomes still more restricted during the fifth cleavage, since the micromeres of the third quartet are again mainly composed of white substance.

The fifth cleavage, dextrotropic in all the cells, produces the third quartet, each cell of which is considerably smaller than the corresponding basal (Fig. 19). Of these cells 3d is much the largest, and is usually composed entirely of white material, while 3a, 3b and 3c usually, perhaps always, receive a certain amount of pigment. At the end of the cleavage the macromeres rapidly diminish in apparent size, evidently owing to their passing more deeply into the egg, and the color-pattern becomes more or less confused, though A, B and C still show the greatest amount of pigment, while D distinctly shows a white area on the side turned towards X, where 4d is subsequently formed. I have not been able to observe the formation of the entire fourth quartet satisfactorily, either in the opaque living object or in preparations. I can however state positively that as seen in surface-view of the living egg, 4d is very small (smaller than 3d and very much smaller than 2d) and appears pure white (Fig 19,a). I have been unable to determine whether the white material of this cell is derived from that of the original lower white area; though, as will appear hereafter, the experimental evidence indicates that such is the case. At this period the four basals appear much smaller, having evidently retreated into the interior.

Beyond this point it is not necessary at this time to trace the cleavage. The foregoing observations clearly show that, *in Dentalium the freshly discharged egg, prior to maturation or fertilization, shows a definite segregation of visibly different materials which accurately foreshadows a corresponding distribution of these materials among the blastomeres during cleavage.* Of the three zones of material superficially visible in the living egg, the upper one (upper white area) is allotted to the first three quartets of ectomeres, apparently in equal amount in each quadrant; the middle pigmented zone is mainly allotted to the four basal entomeres, though a portion also passes into ectomeres of the second and third quartets; while the lower zone (lower white area) certainly passes mainly into the first somatoblast, 2d, or X, probably in part into the second somatoblast, 4d, or M, and possibly in part into the left posterior micromere, 3d, of the third quartet. This agrees in general with the history of the



zones visible in the living egg of *Myzostoma*, as observed by Driesch ('96), where the lower polar area is represented by a green substance, the upper one by a reddish material, and the pigment zone of *Dentalium* by a zone of clear protoplasm. It is important not to confuse the above-described distribution of white and pigmented material with that of protoplasm and deutoplasm. As shown on a preceding page the upper white area is not, like the lower one, free from yolk; and in point of fact all the cells contain a large amount of yolk. The pigment-pattern is only a visible expression in the living object of a distribution of specific materials that can only in part be distinguished in sections.

We may now briefly consider the main outlines of the larval development. In warm weather the embryos become ciliated at about the ninth or tenth hour, and at the end of twenty-four hours are well developed trochophores that swim very actively at the surface, progressing in a spiral curve and rotating from right to left as seen from the side. At this period (Fig. 29) the body is of a blunt spindle-shape, encircled at the equator by a very broad prototroch composed of three principal rows of large trochoblasts which bear three corresponding rows of powerful cilia completely encircling the body and leaving no dorsal gap (as is also the case in *Patella*). The pre-trochal and post-trochal regions, while somewhat variable, are at this period nearly similar in form and size, being roughly conical and rounded at the tip. The pre-trochal region is wholly covered with very short vibratile cilia and bears at its apex a very long and well-defined tuft of flexible, but not vibratile, flagelliform sensory hairs. In total preparations, or in longitudinal sections, it may be seen with great clearness that the apical tuft is borne upon a large and definitely circumscribed apical thickening or plate, sharply marked off from the surrounding cells. The post-trochal region is not ciliated, but bears at its posterior extremity a small bunch of sensory hairs, which differ from those of the apical tuft in being quite stiff, and radiating from the common point of attachment. The alimentary canal at this period forms a closed sac divided into two chambers, into one of which at a slightly later period opens the mouth, formed immediately below the prototroch, but

the anus does not yet exist. The post-trochal region already shows the mantle fold and the beginning of the shell-gland. On either side the gut may be seen an irregular mass of small cells which I believe to represent the cœlomesoblast, though I have not yet traced them to the pole-cells. These masses are not to be confounded with two masses lying further forward that are proliferated off from the ectoblast in two symmetrically placed lateral areas in the pre-trochal region and perhaps represent a part of the pædomesoblast (ectomesoblast) or perhaps the foundations of the cerebral ganglia. These areas, which are figured by Kowalevsky ('83, Figs. 32, 37, 55) are shown in the lobeless embryos (Figs. 33, 40).

The ensuing changes take place very much more rapidly in the Naples species (*D. entalis*) than in the northern form studied by Lacaze Duthiers ('57), which is probably due in a measure to the higher temperature. By the 30th hour the post-trochal region has considerably elongated and the pre-trochal region is somewhat diminished (Fig. 30). In the course of the ensuing twelve hours the pre-trochal region wholly disappears from view, being withdrawn into the interior, while the post-trochal region becomes still more elongated and the larva sinks to the bottom, where it swims only sluggishly. About this time the body becomes surrounded by an extremely delicate hyaline shell into which the greatly diminished prototroch can be withdrawn; and by the end of the second day the foot appears on the median ventral side. By the end of the third day the foot has become a large protrusible organ, trilobed towards the free end, and the prototroch is still smaller (Fig. 31, which closely agrees with Lacaze's Fig. 1, Plate VIII). In many cases the metamorphosis is complete by the end of the fifth day, the prototroch having disappeared, the otocysts and pedal ganglia being clearly visible, and the young *Dentalium* assumes the condition figured by Lacaze on Plate 8, Figs. 2, 3—a larva of 20-25 days (!).

Many details have been omitted from the above account that have already been described in the well-known memoirs of Lacaze Duthiers ('57) and Kowalevsky ('83). Many others will require for their full elucidation much more extended study than

I have thus far been able to devote to the subject. The greatest gap in my work thus far is the failure to trace the connected history of the mesoblast, which can only be done by a complete study of the cell-lineage. This presents considerable obstacles owing to the difficulty of obtaining good total preparations at every stage (the eggs and embryos stain diffusely in most dyes, and the great abundance of deeply staining yolk in all the cells renders it difficult to get clear pictures), and my time was so taken up with the study of the living material that I had not opportunity to work out a really satisfactory method. For sectioning the best results were given by sublimate-acetic, the sections being stained with thionin, which gives a sharp nuclear stain without coloring the yolk. The best total preparations were obtained by mounting in balsam without staining. Apart from the technical difficulties, the object is itself difficult, in the earlier larval stages on account of the difficulty of distinguishing between mesoblastic and entoblastic elements in the crowded mesentoblast-mass, in the later ones by reason of the complication introduced by the folding of the mantle and the shell-gland.

#### EXPERIMENTAL PART.

The ease with which the eggs of *Dentalium* may be operated recalls the remark of Lacaze that "L'embryon du Dentale est un de ces exemples faits pour l'étude du développement" ('57, p.196). For experimental purposes however it presents certain difficulties that should carefully be borne in mind in considering the results of the operations. First, there is a certain amount of variation, not wide but still noticeable, in the size of the eggs and the resulting larvae, and in the relative size of the polar lobe and of the blastomeres during the cleavage-stages. Second, a certain proportion of the entire eggs sooner or later develop abnormally, which results in an increasing mortality from day to day. Third, and most important, the percentage of monstrous forms, and the mortality, is always very large in the development of egg-fragments and of isolated blastomeres. This is undoubtedly due in part to the abnormal conditions under which the larvae are placed in the aquarium, in part to the shock of the operation, and in part



to the changed condition of surface-tension in the dwarf embryos and larvae, as is shown by the readiness with which they disintegrate. (I have several times seen an actively swimming dwarf larva suddenly fly to pieces on coming in contact with an obstacle or even with the surface of the water.) For these reasons, despite the great ease with which the eggs may be operated, it is difficult to base trustworthy conclusions regarding the more special features of the egg-localization on the defects observed in the individual partial larvae. I have therefore in the following work restricted my account in the main to the results that appear with unmistakable clearness, and appear in so large a proportion of the larvae as to remove all reasonable doubt. Beyond this, owing to the importance of following the development of the living larvae as far as possible, the number preserved for sectioning was not very large, and the technical difficulties indicated above, in case of the normal larvae, here appear in aggravated form. This explanation is necessary to account for certain obvious gaps in the work, which I hope to fill out by further investigation, especially those relating to the mesoblast, regarding which I can at present offer only somewhat provisional conclusions.

### III.

#### EFFECT OF REMOVING THE POLAR LOBE.

(a) *General History of the lobeless Larvae.*—During the trefoil stage of the first cleavage the polar lobe may easily be removed, wholly in part, by means of a fine scalpel. Complete removal of the lobe produces a highly characteristic and constant, though in one respect very unexpected, result. Exactly as Crampton earlier found in *Ilyanassa*, the egg continues to segment after this operation quite symmetrically, in a manner similar to the normal cleavage of such forms as *Patella* or *Lymnaea*, giving rise by typically alternating spiral cleavages to successive symmetrical quartets of micromeres (Figs. 20-26). These cleavages differ constantly in two respects from the normal, namely, that (1) *no trace of a polar lobe is formed at either the second or the third cleavage*, and (2) *the members of the D-quadrant are no*

*larger than the others.* Correlated with this is the fact that these embryos show no lower white area, all the basal quadrants being uniformly pigmented over the lower pole (Figs. 23, 24), which sometimes shows a large opening into the cleavage-cavity (Fig.

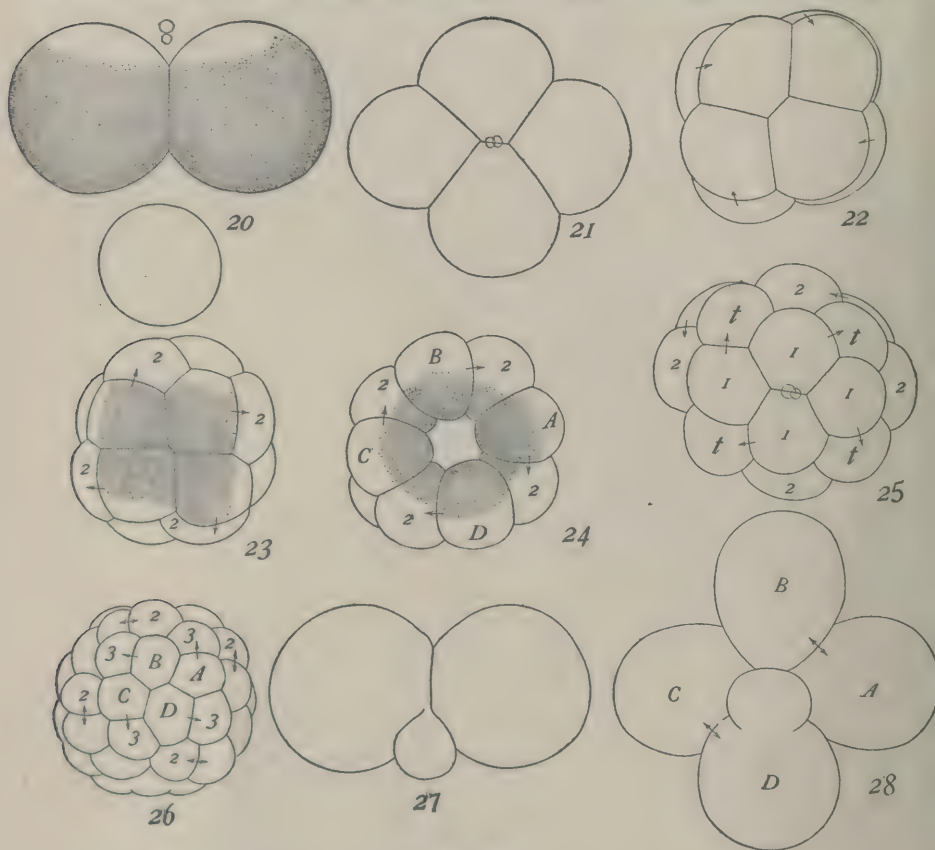


FIG. IV.

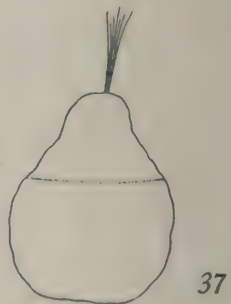
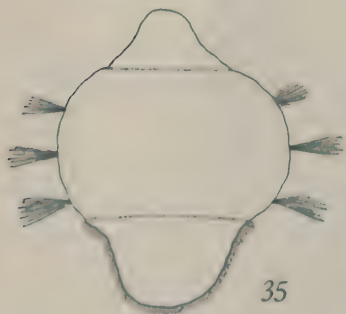
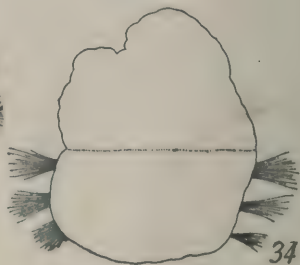
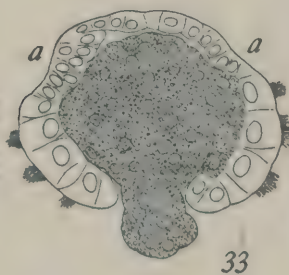
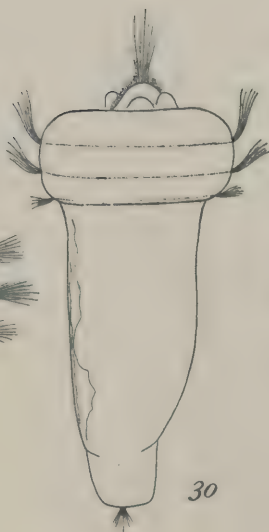
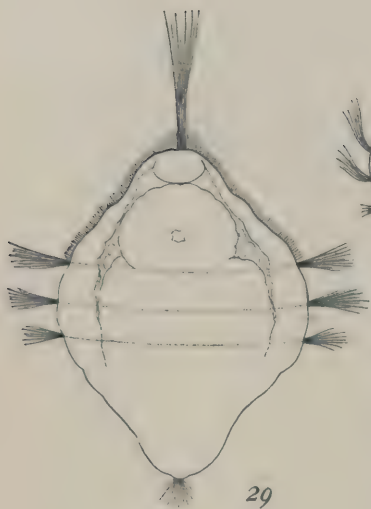
*Cleavage after Removal of the Polar Lobe.*

20, Two-cell stage and polar lobe after removal of the latter; 21, four-cell stage of same, from upper pole; 22, eight-cell stage of same, from upper pole; 23, sixteen-cell stage of lobeless embryo from lower pole, symmetrical second quartet; 24, similar view of the same stage, open type; 25, sixteen-cell stage, from upper pole; 26, lobeless embryo from lower pole, after formation of the third quartet; 27, second cleavage, from the side, of egg from which about three-fourths of the first polar lobe had been removed; 28, a similar form, viewed from the lower pole, after removal of about one-half of the first lobe.

24). The embryos gastrulate and develop with great regularity into larvae that swim in the same characteristic progressive spiral course as that of the normal ones. These larvae (Fig. 32) differ from the normal ones in two obvious respects, namely, (1) *the post-trochal region is absent, or represented only by a smoothly rounded surface from which no outgrowth takes place*, and (2) *they show no trace of an apical organ.* The first of these results fully accords with expectation; for studies in cell-lineage have shown, both in annelids and in mollusks, that in forms possessing a typical trochophore larva the ectoblast and mesoblast of the post-trochal region are mainly derived from the two somatoblasts, and I have shown that the first of these cells is certainly and the second probably, derived mainly from the polar lobe (or lower white area). The second result, on the other hand, is astonishing, since the region that has been removed is diametrically opposite to that from which the apical organ develops; but a large number of operations have not shown one exception in this respect and the most convincing corroborative evidence is afforded by other experiments presently to be described.

The structure and subsequent history of these larvae is very widely different from that of the normal forms. As the cleavage advances the symmetrical cells of the second and third quartets close in around the lower pole, frequently followed in greater or less degree by the cells of the prototroch; and after the gastrulation this region (the posterior region of the larva) becomes somewhat expanded, so that the larva assumes a pyriform shape, actively swimming with the narrower end in front, and rotating from right to left like a normal larva. The narrower anterior region is uniformly covered with fine vibratile cilia which are slightly longer near the anterior pole (as in a normal larva—Fig. 32); but an examination of more than fifty such larvae failed to show a single case in which a true apical tuft was present. Sections and total preparations reveal the remarkable additional fact that in such larvae, at least in many cases, no apical plate is formed, though the lateral areas of proliferation, referred to above, are present, as shown in Fig. 40, a, a. In a few cases I have found a somewhat vague thickening at the apical pole,





## FIG. V.

*Normal Metamorphosis and lobeless Larvae.*

(Excepting Fig. 33 these figures were drawn from living larva, the cilia being added from formol preparations and the inner outlines from specimens mounted in balsam.)

29, Normal trochophore of 24 hours (a rather large specimen); 30, normal trochophore of 32 hours; 31, normal larva of 72 hours, showing foot and shell; 32, larva of 24 hours, after removal of first polar lobe; 33, vertical section of lobeless larva of 24 hours, showing entoblast-plug protruding through the blastopore; 34, larva of 72 hours, after removal of first polar lobe; 35<sup>1</sup> larva of 24 hours, produced from a form like Fig. 28, after removal of about half the polar lobe; 36, larva of 24 hours, after removal of second polar lobe; 37, CD half-larva, after removal of second polar lobe, 24 hours.

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<sup>1</sup> This figure has been turned upside down by the engraver.

but never one that could be mistaken for a typical apical plate. In others, however, the apical ectoderm does not differ from that by which the whole pre-trochal region is surrounded. I feel justified therefore in the statement that the lobeless larvae typically fail to develop the apical organ at any period, individuals having been reared up to the fourth day, when the metamorphosis of the normal larvae was well advanced. (*Cf.* Figs. 31 and 34.) During the development, probably owing to the deficiency of material present in the D-quadrant, the trochoblasts often become more or less displaced towards the posterior pole, and in greater or less degree lose their regular arrangement. In many specimens nevertheless the typical prototrochal belt of three rows of cilia is formed (Fig. 34), though even in these the rounded posterior region often also bears patches of cilia. In others no definite belt can be made out, and such individuals often give the appearance, when alive, and even after being killed with formol, of being ciliated over the whole posterior region. In preparations, however, the cilia of such forms may almost always be seen to be arranged in patches, leaving non-ciliated regions between them, which are doubtless occupied by cells derived from the second and third quartets. It is probable, therefore, that the appearance of uniform ciliation is misleading, and is caused by the confusion of separate tufts lying at different levels. In cases where no displacement of the trochoblasts occurs, the posterior region is covered by cells derived from the second and third quartets.

As the development proceeds there is no attempt to regenerate the missing post-trochal region or apical organ, and the later history of these larvae differs totally from that of the normal ones. The pre-trochal region shows an increase, instead of a decrease, in size, and is not withdrawn into the interior, but gives rise to a more or less irregular vesicular structure directed forwards as the embryo swims. Such larvae were reared until the beginning of the fourth day (Fig. 34), after which they invariably became more and more irregular and finally disintegrated. At this period they present a most remarkable contrast to the normal control larvae of the same age. There is still no trace of a post-trochal region, no shell, no foot, and no apical



organ. Sections show that these larvae have formed no shell-gland, no mantle-fold, and apparently also no mouth.

The foregoing account applies to the great majority of the lobeless larvae; but occasionally an apparent exception occurs, the careful examination of which only serves to confirm the rule. In these exceptional cases a more or less reduced post-trochal region appears to be present, and one individual was obtained that in life seemed to possess this region in a fully developed condition. Sections of these embryos show, however, that what appears to be a post-trochal region is in reality a plug of entoblast cells, projecting through the blastopore-region, that arises through defective gastrulation (Fig. 33). Such embryos sometimes show towards the upper pole a much larger cleavage-cavity than in the normal form,—obviously a result of the failure of the entoblast-cells to invaginate completely. This is conspicuously shown in the larva, referred to above, which appeared to have a fully developed post-trochal region. This larva, cut into longitudinal serial sections, shows very clearly the failure of the entoblast-cells to invaginate properly, a large space being left in the upper hemisphere above the archenteron. For this very reason this larva showed very clearly, both as a total preparation and after sectioning, the entire lack of an apical organ.

The foregoing observations fully establish the conclusion, I believe, *that the material of the polar lobe is indispensable for the formation of the post-trochal region and the apical organ, and as shown beyond they give considerable reason for extending this conclusion also to the cœlomesoblast.* That the failure to produce a normal larva is not due to the lack of sufficient material, is conclusively shown by several additional facts. First, in *Patella* the D-quadrant is no larger than the others, yet a post-trochal region is formed that is relatively as large as in *Dentalium*. Second, as will be described in Part V, much smaller larvae, possessing all of the typical parts, may be produced from fertilized egg-fragments. Third, the same conclusion is afforded by the history of isolated blastomeres, which also fully corroborates the results obtained by removing the polar lobe from an entire egg. If in the 2-cell stage the two blastomeres, AB and CD, be sep-

arated, both continue to segment for a time as if still forming part of an entire embryo, the second and third polar lobes forming in normal fashion in the CD half; but in the end both completely close, gastrulate, and form actively swimming larvae. The two larvae agree in possessing a closed, though often somewhat asymmetrical or confused prototroch, but otherwise show the following characteristic and constant differences. The AB (smaller) larva, closely resembles, except in size, that derived from an entire egg from which the polar lobe has been removed, invariably lacking a post-trochal region and apical organ (Fig. 46). The CD (larger) larva, on the other hand, possesses both these structures, both of which may be as large as in a whole embryo (Figs. 42-45). These larvae vary greatly in form, but in general are asymmetrical and, as may be seen by a comparison of Figs. 45 and 29, possess a post-trochal region that is almost invariably relatively too large, and a pre-trochal region relatively too small as compared with a normal larva. As in the AB half, the prototrochal cilia frequently show a confused arrangement, the regular rings of the normal larva being more or less broken up. In like manner, if the four blastomeres of the 4-cell stage be isolated, only the larva from the D (largest) quadrant develops these two structures (Fig. 47), while those from A, B or C are nearly like those derived from the AB half, though only half as large (Figs. 48, 51). Like the CD  $\frac{1}{2}$ -larvae the D  $\frac{1}{4}$ -forms are variable in form; but whenever they complete what may be considered their normal development they show the post-trochal region very much too large, and the pre-trochal region much too small (Fig. 47).

All these larvae show a very high mortality, but I have kept the  $\frac{1}{4}$ -larvae as late as the beginning of the fourth day (Fig. 51), and the  $\frac{1}{2}$ -larvae nearly as long. The smaller larvae (the AB half, or the small quarters) show a greater tenacity of life, swim more actively, and become less irregular than the larger ones. In the end, however, all the forms become irregular and finally wholly disintegrate, without producing normally formed trochophores or regenerating the missing structures. The CD  $\frac{1}{2}$ -larvae of 24 hours sometimes approach the form of normal larvae of the same age, though always showing the false proportions of the pre-tro-

chal and post-trochal regions described above. Like the AB halves and the  $\frac{1}{4}$ -larvae, they often swim actively at the surface, rotating in the same way as an entire larva; though the progressive movement is almost always slower and less regular than that of the smaller halves. These forms, however, seem to live no longer than the less regular ones, and in spite of every precaution they become more and more irregular and finally disintegrate in the same aquaria containing the normally developing whole larvae. Those that lived to the end of the second day invariably became monstrous in form and showed no resemblance to a normal larva. The history of the AB halves or the smaller quarters in general very closely resembles that of the lobeless larvae, the pre-trochal region enlarging, becoming irregular, and finally disintegrating, often while the embryo is still actively swimming by means of the trochoblasts, which, as Fischel has observed in case of the swimming cells of ctenophores, are most tenacious of life of all the cells.

The relative volumes of protoplasmic substance contained by these various forms of larvae, may be determined either by measuring the volumes of the blastomeres after isolation by means of calcium-free sea-water, or by measuring the polar lobe and estimating the other volumes, the two methods giving fairly consistent results. It should be remembered, however, that both the whole eggs and the relative size of the polar lobe (and hence of the blastomeres) vary somewhat, both in the eggs produced by a single female, and to some extent in those produced by different females. I observed one lot of eggs, for instance, the greater number of which produced lobes considerably smaller than usual. Measurements of the lobe in typical average trefoils give a value ranging from one-fifth to one-sixth that of an entire egg. A typical case gave a volume of 0.18 for the lobe, from which the other volumes are as follows:

Entire embryo.....	1.00
Embryo without polar lobe.....	0.82
CD $\frac{1}{2}$ embryo.....	0.59
AB $\frac{1}{2}$ embryo.....	0.41
D $\frac{1}{4}$ embryo.....	0.385
A, B or C.....	0.205



Since the CD  $\frac{1}{2}$  larva is less than  $\frac{3}{4}$  and the D  $\frac{1}{4}$  larva less than  $\frac{1}{2}$  the volume of the lobeless embryo, yet both produce apical organ and post-trochal region, the conclusion is unavoidable that *the failure to form these structures after removal of the polar lobe must be due to a qualitative and not a quantitative difference*; in other words, the material of the lobe must be specifically different from the remaining material, and as such is the determining cause of the development of the structures in question.

The above conclusion is fully sustained by the effect of cutting off only a part of the polar lobe. In such embryos during the second and third cleavages the polar lobe is correspondingly diminished in size (Figs. 27, 28), and the D-quadrant is too small by the same amount. Such eggs produce larvae with a corresponding reduction in the post-trochal region (Fig. 35) and these larvae sometimes possess, sometimes lack, the apical organ. It is not improbable therefore that further experiments of this kind may show a localization, within the polar lobe itself, of the determining materials of the apical organ and of the post-trochal region. This experiment adds to the foregoing the important result that after the polar lobe has formed there is a direct quantitative relation between the amount of specific material it contains and the size of the post-trochal region, there being apparently no regulative process in the later stages (though I have not yet sufficiently examined this latter point). As will appear in Part V, *this conclusion does not apply to the material of the lower polar area before the formation of the lobe.*

(b) *The mesoblast question.*—We may now consider what is in some respects the most interesting, as it is certainly the most difficult, of the questions relating to the lobeless larvae, namely, that of the mesoblast. The fact that certainly the first and probably the second somatoblast is derived mainly from the substance of the polar lobe, and that after the removal of this substance the post-trochal region fails to develop, suggests that the material of the cœlomesoblast as well as of the ectoblastic structures, is localized in the polar lobe and hence in the original polar area. In point of fact

Crampton ('96) in his interesting paper on *Ilyanassa*, found that after removal of the polar lobe the second somatoblast (4d) differs from the normal not only in being no larger than the other members of the quartet, but also in texture, being filled with yolk-spheres instead of being mainly composed of clear protoplasm as in the normal, and it also lies at first at the surface, exactly like 4a, 4b and 4c. This observation I can confirm from a reëxamination of the original preparations, kindly placed at my disposition for this purpose by Dr. Crampton. He found further, that the larvae produced from such eggs lacked the mesoblast-bands present in the normal larva, 4d apparently entering, like its fellow-members of the same quartet, into the formation of the archenteron.

This highly interesting result, which has attracted considerable attention, was based on the examination of total preparations only; and the desirability of a more adequate study of the matter by means of sections has long been obvious. I have accordingly given especial attention to this point as far as my material would allow; but must admit that neither in point of abundance nor of fixation is this material quite adequate for the full investigation of the question, which indeed would demand a complete study of the cell-lineage, both in the normal and in the lobeless forms. Nevertheless such evidence as I have obtained is distinctly in favor of the correctness of Crampton's result.

The mesoblast may be most clearly seen in the normal larvae in cross sections through the region of the prototroch, where the gut shows two chambers and the complication produced further back by the shell-gland and mantle-folds are not present. In such a section (Fig. 38) the gut appears in the form of two distinct chambers, the wall of the ventral one being a little further back intimately connected with the stomodæal invagination (Fig. 39) though its cavity does not yet appear to communicate with the outside. The walls of both chambers are composed of large cells, more or less columnar and radially disposed, completely filled with yolk-spheres (as are all the cells at this time) and with large nuclei. On either side is a loose group of much smaller cells with small nuclei, that appear irregular or often spindle-

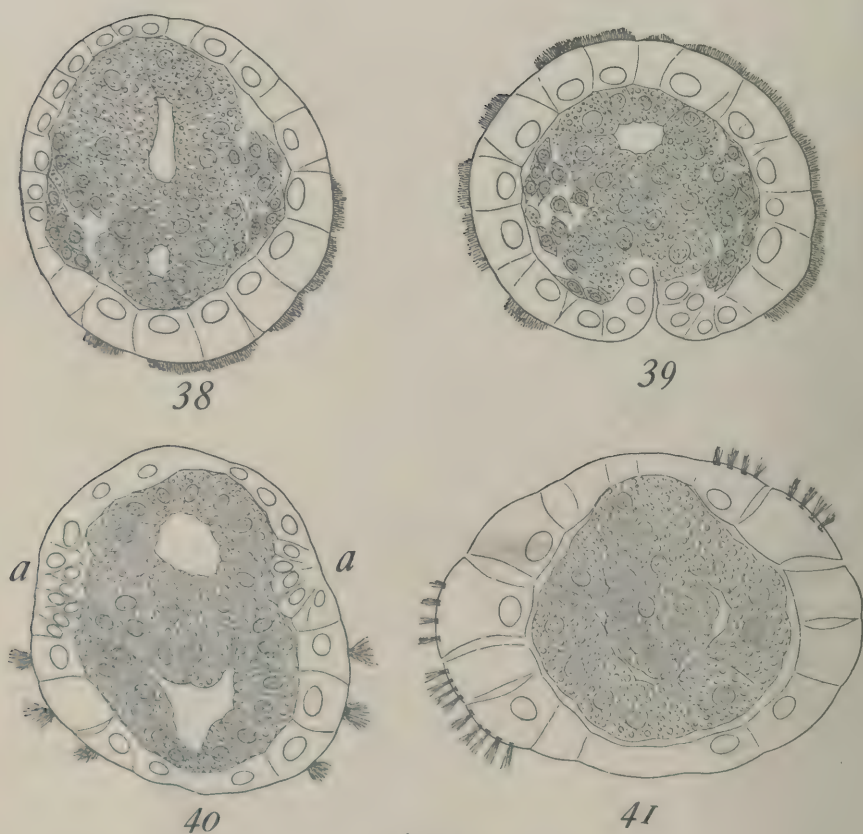


FIG. VI.

*Sections of normal and lobeless Larvae.*

(Each of these is drawn from a single section, supplemented by a few details from the two adjacent sections of the series. The deutoplasm is only shown in the entoblast and mesoblast.)

38, Slightly oblique cross-section of normal larva, 24 hours, just anterior to the mouth; 39, cross-section through the mouth; 40, vertical section of lobeless larva, 30 hours; 41, cross-section through prototroch-region of lobeless larva, 48 hours.



shaped. There can, I think, be no doubt that these are mesoblast cells,<sup>1</sup> though I have not determined whether they are the products of the second somatoblast, 4d, or arise from another source. A possibility of error on this point is given by the fact, already referred to, that just anterior to the prototroch on either side are two lateral ectoblastic areas of proliferation (of unknown significance) that may contribute to the small cells in question. In any case *these lateral masses of mesoblast fail to appear in the lobeless embryos of corresponding age or older.* In the earlier stages, of which Fig. 33 is an example, it is impossible to determine this point with any degree of certainty, owing to the crowding together of the entoblast cells in a compact mass in which frequently no cavity can be seen. In later stages, however, both longitudinal and transverse sections give pretty clear evidence that the small mesoblast-cells are either wholly absent or very few in number. Fig. 40 is from a complete series of longitudinal sections of a lobeless embryo of 30 hours. This shows the gut as a two-chambered sac directly applied to the ectoblast with no sign of smaller cells between them, though both the anterior ectoblastic areas of proliferation are shown (*a, a*). It might well be supposed that the small cells are present in a different plane, as would be the case in Fig. 38 if cut in the sagittal plane; but their absence appears no less clearly in cross-section, as shown in Fig. 41 (from a complete transverse series). This embryo of 48 hours swam actively and normally. Though not so well fixed as the preceding one, it clearly shows the gut as a simple sac, enclosing a single cavity that opens at the posterior pole and anteriorly is nearly filled with a thickening bulging inward from the wall at one side. I am quite sure that no mesoblast-cells are present in this embryo unless at the extreme anterior end, where the layers are cut tangentially and cannot be clearly analyzed. The sections of this embryo clearly show further

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<sup>1</sup> The relations as figured by Kowalewsky ('83, Fig. 48) in the Marseilles species are essentially similar to those here shown, except that the mesoblast-cells are shown very much larger and fewer. This is stated to be from a larva of 24 hours, but probably represents a relatively earlier stage of development than mine. Compare the mesoblast-cells in Kowalewsky's Fig. 66, from a larva of 38 hours.

the absence of any structure comparable with the foot, mantle-folds, shell-gland, or mouth (unless the posterior opening can be so considered) though all these structures are present in the normal control embryos. The absence of an apical organ is shown as in other series, by the two from which Figs. 33 and 40 are taken.

I would not speak too positively before examining additional material, for in some of the other series a few small cells appear that may be of the same nature as those seen in the normal embryos, though they are far less numerous; yet the foregoing evidence is sufficient to create a strong presumption that Crampton's result was correct. Crampton showed due caution in guarding against the conclusion, from his observations, that the polar lobe "contains prelocalized mesoblast material," being probably influenced by the fact that in *Ilyanassa* the lobe appears to be composed mainly of deutoplasm. He only concluded "that the presence of the yolk mass in the cell D may be the stimulus which causes that cell to act differently from the other macromeres, A, B and C" ('96, p. 14). I believe, however, the facts brought forward in this paper render it probable that the polar lobe (and hence the cell D) does in fact contain a specific kind of cytoplasm which, if not actually "prelocalized mesoblast-material" is the direct and necessary antecedent of that material.

#### IV.

##### LOCALIZATION OF THE APICAL ORGAN AND ITS CORRELATION WITH THE POST-TROCHAL REGION.

The failure of the AB half-larva to produce an apical organ, though wholly consistent with the history of the lobeless embryos, was to me a surprising fact; for the development of this organ in other forms indicates that all of the four quadrants contribute to its formation; and in point of fact I had found in *Patella* that not only do both the AB and CD halves produce an apical organ, but also any of the  $\frac{1}{4}$ -embryos, and even any isolated micromere of the first quartet. I therefore turned with much interest to a more detailed examination of the localization of this organ in *Dentalium*; and this involved the inquiry whether the correla-

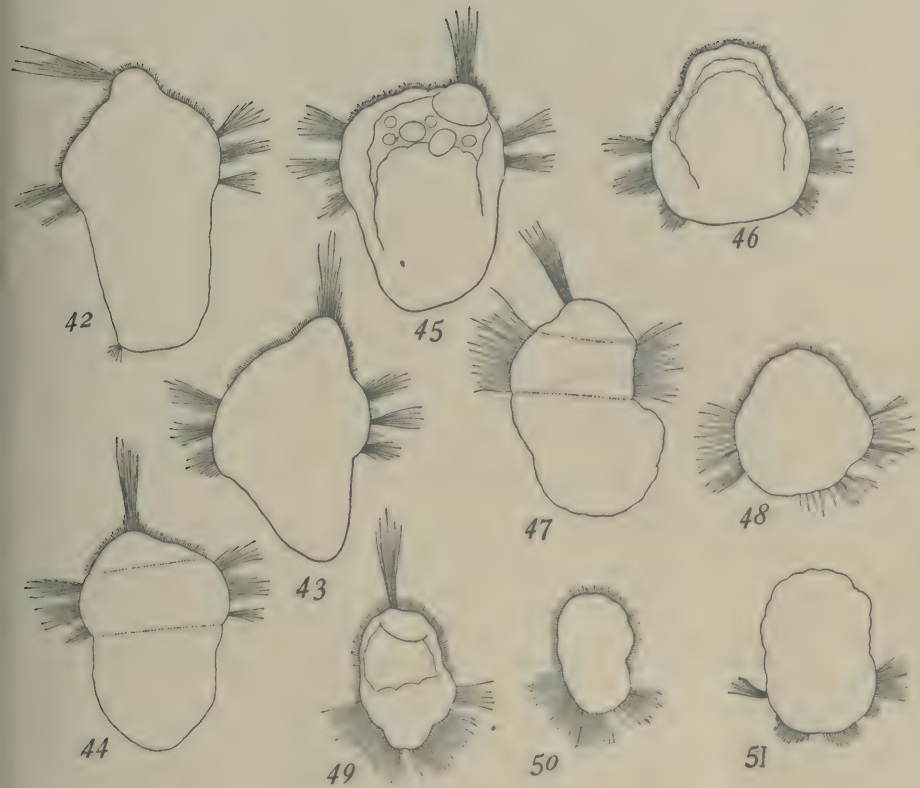


FIG. VII.

*Larvae from isolated Blastomeres.*

42, 43, 44. Various forms of larvae from isolated CD halves, 24 hours; 45, 46, twin larvae from the isolated CD and AB halves of the same egg, 24 hours; 47, larva from isolated D-quadrant, 24 hours; 48, larva from isolated C-quadrant of the same egg, 24 hours; 49, larva from isolated posterior micromere, 1d, of 8-cell stage, 24 hours; 50, larva from isolated micromere, 1c, of the same egg, 24 hours; 51, one-fourth larva from one of the small quadrants (A, B or C), 72 hours.



tion between apical organ and post-trochal region is direct or indirect—*i. e.*, whether the development of the one depends on that of the other, or whether the development of the two is only connected through their common relation to the polar lobe. Further experiments conclusively show that the latter is the case; for in several ways larvae may be produced that possess the apical organ but lack the post-trochal region. My first experiment to test this consisted in the isolation, separately, of the four micromeres of the first quartet (1a, 1b, 1c, 1d), which may easily be effected by means of Herbst's calcium-free sea-water. The result of this experiment, several times repeated, is that while all four of these micromeres may develop into actively swimming ectoblastic embryos, *the one derived from the D quadrant (1d), and this alone, develops an apical organ* (Figs. 49, 50). All of these four small embryos are of approximately the same size, ovoidal or somewhat pear-shaped in form, with a group of active trochoblasts at the larger (posterior) end. The anterior region is covered with fine cilia (as in the AB  $\frac{1}{2}$ -larva or the A, B or C  $\frac{1}{4}$ -larva); but only the 1d larva bears in addition the characteristic apical tuft, which is nearly or quite as large as in a whole embryo, and is borne upon the usual ectoblastic thickening or apical plate. None of these larvae gastrulate or develop a post-trochal region; from which it follows that *after the completion of the third cleavage not only is the development of the apical organ independent of that of the post-trochal region, but at this time the posterior micromere of the first quartet, 1d, is already definitely specified for the formation of that organ*, independently of its relation to the remainder of the embryo. The result of isolating the cells of the 4-cell stage is entirely in harmony with this, as already mentioned. The A, B or C  $\frac{1}{4}$  develops into a closed pyriform larva swimming normally with the smaller and turned forwards, but entirely devoid of apical organ or post-trochal region (Fig. 48). The D  $\frac{1}{4}$ , on the other hand, though often distorted, shows typically the apical organ, and an exaggerated and usually irregular post-trochal region. (Fig. 47.) This result is in striking contrast to the fact, mentioned above, that in *Patella*, each of the quadrants, whether of the 4-cell stage or of the first quartet, may develop an

apical organ. The only conclusion that can be drawn from this contrast is that the definitive basis of the apical organ is more closely localized in *Dentalium* than in *Patella*, being concentrated in a single cell.

The above results prove that the determination of the development of the apical organ takes place at some period between the first and the third cleavages. Further experiments fix the period of determination still more nearly. If the egg be allowed to advance as far as the second cleavage and the polar lobe formed at that time be removed, the egg continues to segment in a manner indistinguishable from that of an egg from which the lobe has been removed at the time of the first cleavage. *From such eggs arise larva agreeing exactly with those arising after removal of the first polar lobe in every respect save one, namely, that the apical organ is typically present, though this is not invariably the case.* (Fig. 36.) Sections clearly show that the apical tuft is borne upon a very definite apical plate, in striking contrast to the larvae arising after removal of the first polar lobe. It is thus possible to produce at will larvae which lack the post-trochal region and either possess or lack the apical organ; and *the determination of the apical organ is thus proved to be effected during the short period between the first and second cleavages.* Complete corroboration is given by removal of the second polar lobe from the isolated CD  $\frac{1}{2}$  during its first division. The resulting larva resembles that arising from the AB half in having no post-trochal region, but possesses an apical organ as well developed as though the polar lobe had not been removed. (Fig. 37.)

The experiments just described prove, first, that the correlation between post-trochal region and apical organ is due to their common determination by the first polar lobe. The second polar lobe, though apparently precisely similar to the first, has no longer any influence on the apical organ, though it still determines the development of the post-trochal region. It seems impossible to explain these facts, save under the assumption that the first polar lobe contains specific stuffs that are in some manner essential to the formation of both structures, and that during the period

between the first and second cleavages the "apical stuff" (if such a term be allowed) exerts once for all its specific effect. The most natural explanation of this is given by the hypothesis that this stuff moves upward to the apical pole, to be isolated in the large posterior quadrant, D, during the second cleavage, and subsequently in the corresponding micromere, 1d, during the third cleavage. The basis of correlation between post-trochal region and apical organ may thus be sought in the physical association of the corresponding specific stuffs in the first polar lobe, while the specification of the posterior micromere, 1d, is due to the final isolation within it of the "apical stuff."

## V.

### LOCALIZATION IN THE UNSEGMENTED EGG.

The preceding sections are in a measure only preliminary to the present one which includes the most important part of the present paper, namely, the results of experiments on the localization of the polar lobe, and of the structures that it involves, in the unsegmented egg. As has already been stated, the clear substance forming the polar lobe is already visible in the egg prior not only to cleavage, but even to fertilization and maturation. *Experiments on the unsegmented egg show with great clearness that this area possesses in a general way the same promorphological value as the polar lobe itself*, though at this early period the egg possesses a greater regulative capacity than at later stages. The unfertilized living eggs of *Dentalium* may readily be cut in two with the scalpel under the microscope, and the plane of section determined with considerable accuracy not only during the operation but by a subsequent examination of the fragments in which the polar areas are often still clearly visible. As Yves Delage first showed, such fragments when fertilized may segment and give rise to ciliated embryos and in certain cases even to dwarf trochophores. In a considerable proportion of such experiments, both fragments develop. For convenience of description I shall divide them into two classes, including (a) those obtained by horizontal or oblique section, and (b) those obtained



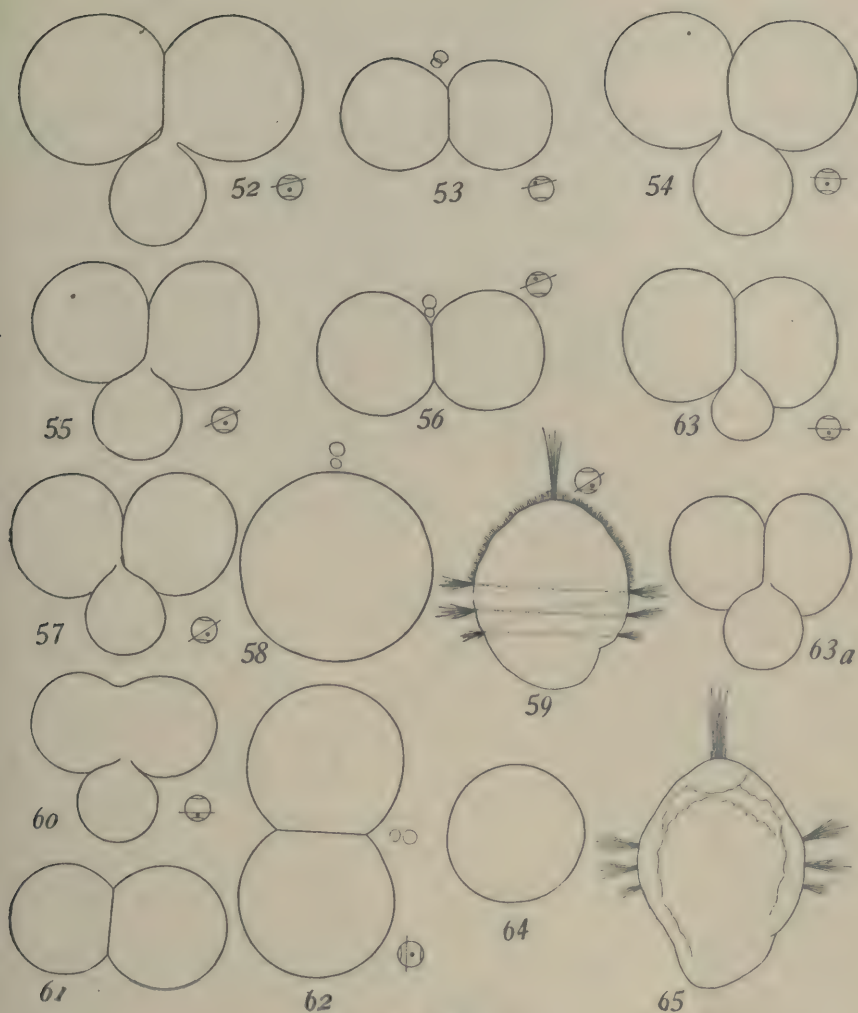


FIG. VIII.

*Development of Egg-fragments after horizontal or oblique Section.<sup>1</sup>*

52, 53, Twins, after oblique or horizontal section near upper pole; 54, trefoil, lower half, horizontal section above equator; 55, 56, twins, after oblique section, larger lower, smaller upper fragment; 57, 58, 59, twins, after slightly unequal oblique section; 57, trefoil from lower fragment, 58, upper fragment (failed to segment), 59, trochophore of 24 hours developed from 57; 60, 61, 62, twins, horizontal section below equator; 60, trefoil, lower fragment, 61, 2-cell stage of same, 62, upper fragment, 2-cell stage; 63, trefoil, lower fragment, horizontal section, polar lobe too small; 63a, trefoil lower fragment, with polar lobe slightly too large; 64, 65, twins, plane uncertain, 64 undeveloped fragment, 65 trochophore, 24 hours.

<sup>1</sup> In these and the following figures the plane of section is indicated by the small accompanying diagram, the fragment studied being marked with a black dot.

by exactly vertical section passing through the axis and bisecting the polar areas.

(a) *Fragments obtained by horizontal or oblique section.*—Under this heading may be grouped all fragments obtained by sections passing in such a plane as to separate the polar areas, so that one fragment contains only the upper, the other only the lower, of these areas. These may be designated respectively as the upper and the lower fragments. Before maturation I have not found it possible to distinguish the upper from the lower fragment; but as soon as the polar bodies form, the upper fragment may be at once identified with certainty, since it alone produces these bodies. I have not thus far observed any difference between the results of horizontal and of oblique sections.

The upper and lower fragments differ in a characteristic way, both in the form of cleavage and in the structure of the resulting larvae; though it should be added that this appears most clearly in the cleavage-process, since many of the embryos die before reaching the trochophore stage, and many of the remainder become wholly monstrous in form. Nevertheless the main result is given with great consistency by a comparison of the larvae. This contrast is especially striking when two fragments from the same egg are compared; and within rather wide limits it is independent of the plane of section and the size of the piece, certainly as far as the form of cleavage is concerned, and apparently also as regards the larval type. Whether large or small the upper fragment forms the polar bodies in normal fashion, and in many cases *segments in essentially the same way as an egg from which the polar lobe has been removed*. The first cleavage takes place without the formation of a polar lobe and is invariably equal (Figs. 53, 56, 62, etc.), and the same applies to the second cleavage. Frequently the two pairs of cells shift during or after the second cleavage, so as to produce a "cross-form," the succeeding divisions of which are difficult to analyze. In many cases, however, the four cells remain in nearly the same plane; and in such cases the succeeding divisions conform to the regular rule of spiral cleavage, quartets of micromeres being found by alternating dextrotropic and leiotropic divisions. (Fig. 69.)

A considerable proportion of these embryos fail to develop into larvae, breaking up sooner or later into loose groups of cells that perish. Many, however, develop into actively swimming larvae, but these, whether large or small, are never normal trochophores. While showing many variations, and often being more or less irregular in form, these larvae tend in general towards, and sometimes agree precisely with those derived from whole eggs minus the polar lobe, from the AB half, or the A, B or C quarters (Figs. 70, 86). They are in general more or less distinctly pyriform, swimming actively by the long cilia that are more or less irregularly disposed about the posterior enlarged region. A typical case is shown in Fig. 70 (from a preparation, the cilia from the living larva) produced from the upper two-thirds of an egg after exactly horizontal section. The cleavage of this fragment was similar to that shown in Fig. 69. This larva is in every respect closely similar to the lobeless larva, though the pre-trochal region is more expanded than usual, forming a large hollow vesicle enclosing a few loose cells, and with a slight thickening at the anterior pole, but without anything like a true apical organ. The posterior region is filled with a crowded mass of rounded cells. Transverse sections of this larva show that this mass incloses a very small central cavity; but it is impossible to determine whether mesoblast cells are present or not. In a very few cases an apical organ is present in such larvae; but this is so rare that I attribute its occasional presence to the fact that the plane of section was not quite correctly determined, a portion of the lower polar area having been in fact included in the piece. Another possibility is that the specific material of the polar lobe extends so far up into the interior as to be removed by a section that externally passes quite outside the polar area. This interpretation is supported by the fact that in a very few cases, when the upper fragment is considerably larger than the lower one, I have seen the upper fragment form a very small polar lobe.

The development of the lower fragment—*i. e.*, one that includes the lower polar area—differs in a remarkable way from that of the upper one, both in the form of cleavage and in the end-result. Whether obtained by horizontal or oblique sections,



and (within rather wide limits) whatever its size, *this fragment may segment in every detail like an entire egg of diminished size, forming the polar lobe in normal fashion, and may give rise to a dwarf larva nearly or quite normal in form and possessing an apical organ.* The study of a large number of these fragments shows that while there is considerable variation in the size of the polar lobe *it is as a rule of approximately and often exactly, of the correct proportional volume;* and this is true even after a horizontal section that passes quite outside the limits of the polar area. By varying the plane of section it is thus possible to obtain a graduated series of forms leading down from a full-sized embryo to one not more than one-fourth this size, the fragments from the other halves forming a similar series grading in the opposite direction. That the form of cleavage is within wide limits, independent of the size of the piece, is thus strikingly demonstrated. Such a graded series of trefoils and the corresponding equal 2-cell stages, is shown in Figs. 52-60, the last of these showing the smallest one observed. Regulation of the size of the polar lobe sometimes fails however, examples being shown in Fig. 63, where, even after horizontal section, the lobe is too small (this egg produced a larvae possessing an apical organ, but with the post-trochal region greatly reduced), Fig. 63*a*, where it is slightly too large, and Fig. 66, where it is much too large; but these are exceptional. It is hardly possible that this apparent regulation is owing to the fact that the specific polar material extends so far up into the interior of the egg that a section in almost any plane includes the right amount of material to form a normally proportional lobe. Such an explanation is rendered very improbable by the usual failure of the upper fragment to form a lobe even after horizontal section far down in the vegetative hemisphere or after oblique section; and still more improbable by the fact that so many of the fragments form a normally proportioned lobe, whatever be the plane of section. The conclusion therefore appears unavoidable that the size of the polar lobe, and hence of the structures dependent upon it, is subject to a regulative process, from which it follows that the *predetermination of the region of the polar lobe is qualitative, not quantitative, or if*

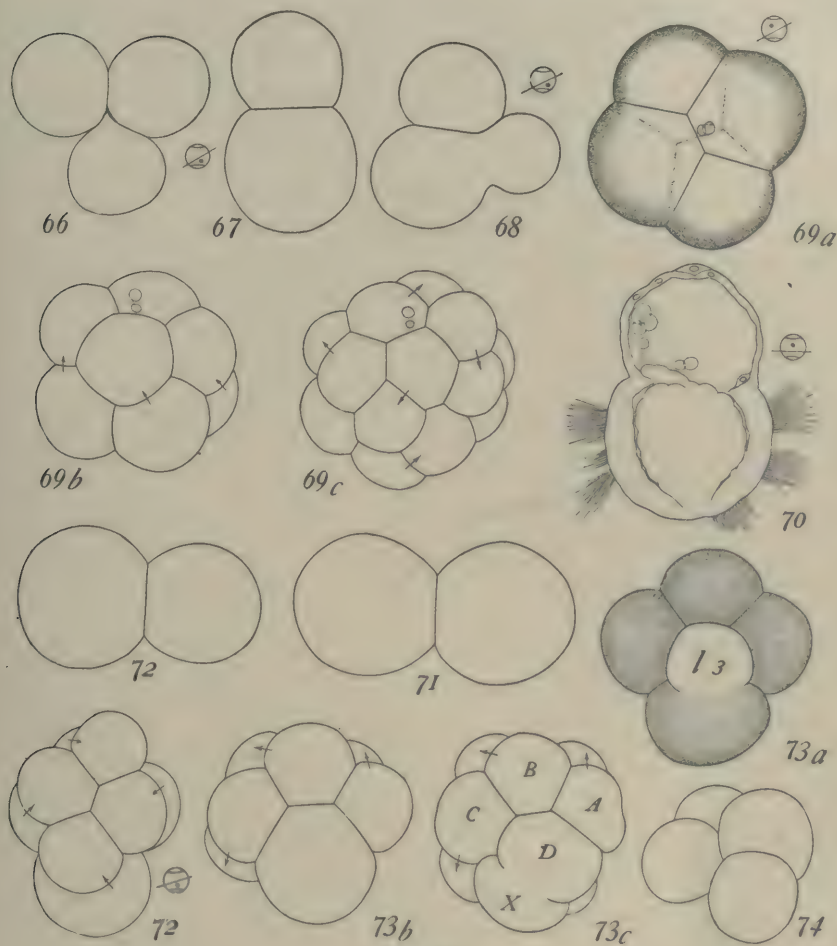


FIG. IX.

*Development of Egg-fragments*

66, 67, Lower fragment, oblique section; 66, trefoil, polar lobe much too large, 67 resulting 2-cell stage, CD half too large; 68, 69a-69c, cleavage of twin fragments, oblique section, 68 trefoil, from smaller lower fragment, 69a-69c, symmetrical cleavage of larger upper fragment, 4-cell to 16-cell stages; 70, larva of 24 hours, from upper fragment of a case exactly similar to 69; 71, 72, twins, oblique section, nearly equal fragments, 71, 2-cell stage of upper fragment, 72 and 72, 2-cell and 8-cell stages of lower fragment; 73, 74, twins, probably oblique section near upper pole, 73, 4-cell stage, 73a, normal third cleavage of lower fragment from lower pole, 73b, resulting 8-cell stage, 73c, beginning of fourth cleavage, formation of first somatoblast.

*quantitative, it is still subject to the operation of a regulative factor that lies behind the topographical distribution of the egg-materials.* This appears to me one of the most significant results that my experiments have yielded.

The embryos may in succeeding stages cleave in every detail like whole eggs. Typical 4-cell stages are shown in Figs. 73a, 78b, 83, 8-cell stages in Figs. 72, 73b, 84, and the fourth cleavage, with the formation of the first somatoblast, in Fig. 73c. Fig. 73a shows the third cleavage with the formation of the third polar lobe. Many individuals were observed showing the formation of the second polar lobe in normal fashion, though none are figured.

The larvae arising from fragments of this type differ as markedly from those derived from the upper fragments as does the cleavage. Although many of the embryos perish, and of those that live many are abnormal, they frequently possess both the apical organ and a post-trochal region; and occasionally a dwarf larva is produced that is essentially similar, except in size, to an entire trochophore. One of the best of these is shown in Fig. 59, which arose from a lower fragment obtained by oblique section, slightly smaller than half the volume of the egg, and including the whole of the polar area. The typical trefoil stage of this larva is shown in Fig. 57; it has exactly the normal proportions, and segmented normally in later stages. This larva is somewhat less pointed posteriorly than the normal, but the whole larvae vary considerably in this regard. It swam in quite normal fashion. Another larger larva from a lower fragment is shown in Fig. 65. The total preparation of this larva shows with great clearness a typical apical plate at the upper pole. Out of a very large number of operations I have obtained altogether not more than five or six such perfect larvae, at least half the embryos dying during the cleavage, and a large proportion becoming abnormal during the later development.

That so large a proportion of the embryos die or develop abnormally is to be expected when we consider the very different mechanical conditions of surface-tension and the like in these small embryos. The fact remains that abnormal larvae may be pro-



duced from lower fragments less than half the size of the egg; and that such larva may possess a typical apical organ when the section passes far away from the apical pole; while in no case does the upper fragment produce a larva that ever approaches the normal form. It may therefore safely be concluded that the dwarf trochophores obtained by Yves Delage ('99) arose from fragments including at least a part of the lower polar area.

The abnormalities observed in larvae from the lower fragments range from only slight defects to wholly irregular and monstrous forms, and thus far do not permit any more detailed conclusions regarding the prelocalization than those stated above. A common defect, illustrated by the pair of twins shown in Figs. 85, 86, is a more or less imperfect development of the post-trochal region, even when the whole lower area is included in the fragment, and sometimes this region appears to be wholly lacking. Much more rarely the apical organ is lacking while the post-trochal region is in greater or less degree developed. Such a case is shown in Fig. 87 (from a preparation), the absence of the apical tuft having been certainly determined in the living larva.

As in the case of the lobeless larvae, the experiments demonstrate that the failure of the upper fragment to produce the missing structures is not due to an insufficient mass of protoplasm; for I have obtained larvae showing the characteristic defects from upper fragments fully two-thirds the bulk of the egg (Fig. 70), and perfect dwarfs from much smaller fragments (Fig. 59). The conclusion is therefore unavoidable that, like the polar lobe to which it gives rise, *the lower polar area contains specific materials that are essential for the formation of the apical organ, and of a post-trochal region*; and that it is these materials that enter into the formation of the polar lobe, as simple observation of the normal development indicates.

(b) *Fragments obtained by vertical section through the axis.*  
—In view of the foregoing results we should expect to find that when the egg is cut exactly vertically, so as to bisect the lower polar area, both fragments should form the polar lobe; and such is in fact the case. The experiments of this type were not very numerous, and only a few cases were obtained in which both frag-

ments developed. I have only one pair of camera sketches to show the polar lobes in such a case (Fig. 75, 76). In both these the lobe is relatively too small, as if produced from insufficient material; but this not always the case (as shown beyond), and it should be remembered that the polar lobe is sometimes too small even in a lower fragment containing the whole of the lower polar area (Fig. 63). Figs. 77a, 78a show a pair, one of which has a lobe of normal proportions; the other is a very nearly normally formed 2-cell stage, though the larger cell is perhaps a trifle too small. Both these produced nearly normally proportioned 4-cell stages (Figs. 77b, 78b). Several other cases, in which only one fragment developed, showed a normal trefoil. These data are somewhat meagre, yet they justify the conclusion, I believe, that after vertical section bisecting the lower polar area both fragments may segment like whole eggs of half size.

The above conclusion renders it probable that by such vertical section two perfect dwarf trochophores may be produced from a single egg, which is apparently impossible when one fragment alone contains the lower polar area. In point of fact, I have never obtained even a single wholly normal larva after such section; but in view of the comparatively small number of successful operations and the very small number of such larvae obtained by section in other planes this is not surprising. A number of larvae from more or less nearly vertical sections is shown in the following figures. Fig. 88 is a nearly normally formed larva with two apical organs, from an oblique section passing outside the lower white area. Fig. 89 is a nearly normal larva from a section that removed a part of the lower area. Fig. 93 is from an exactly vertical section bisecting both areas. In section this larva is closely similar to a normal one, and seems to show that the trochoblasts are as large as in a whole embryo. Fig. 90 is from the smaller fragment after a slightly oblique section bisecting the lower area; a very distinct apical organ is present and also an abnormally formed post-trochal region. Figs. 91, 92 are twins from a slightly unequal vertical section (developed from the respective twin fragments 81, 82), the post-trochal region is lacking in both, while one lacks an apical organ.

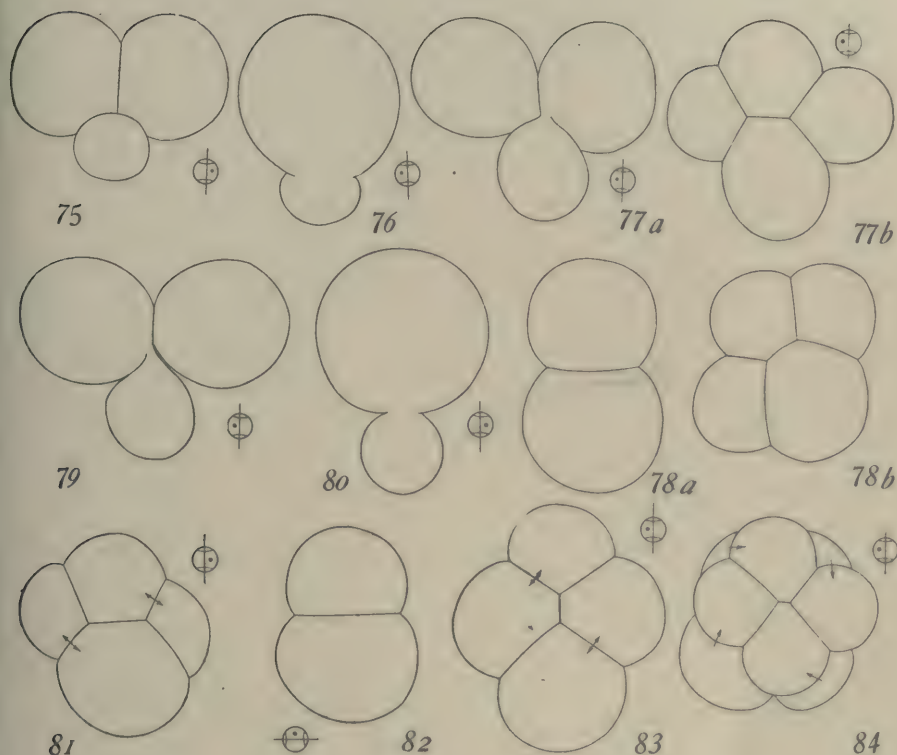


FIG. X.

*Development of Egg-fragments after vertical Section.*

75, 76, Equal twins, respectively in trefoil and polar lobe-formation; lobes too small; 77, 78, equal twins, nearly correct proportions; 77a, 77b, typical trefoil and 4-cell stages of one fragment; 78a, 78b, typical 2-cell, slightly abnormal 4-cell stages of the twin fragment; 79, 80, twins, from a fertilized egg, 79, nearly normal trefoil, 80, the twin, with reduced polar lobe; 81, 82, nearly equal twins, 81, typical 4-cell stage, 82, its twin, nearly typical 2-cell stage; 83, 84, typical 4-cell and 8-cell stages, from upper pole, of the same fragment.



It may be pointed out that not one of these larvae shows a fully developed post-trochal region, though 91 and 92 arose respectively from 2- and 4-cell stages that show nearly the normal proportions and must have been produced from nearly normal trefoils. This may seem to contradict the conclusion, drawn above, that the predetermination of the lower polar area is not quantitative; but a similar reduction sometimes exists in this region when the whole polar area is present (as in Fig. 85), and I do not think a trustworthy conclusion can be drawn without additional data.

I may add that after a large number of unsuccessful attempts I obtained two nearly normal dwarf trochophores from fragments of the unsegmented egg of *Patella*. One of these, which is about half the volume of a normal larva, clearly shows the cells of the prototroch. In the full-sized normal trochophore of *Patella* the prototroch, as may be seen with the greatest clearness in total preparations, consists of a closed principal ring of cells that vary in number (as seen in optical section) from 19 to 21. In the dwarf the cells are more variable in size and less regularly arranged, but on the average as large as in the normal individual; equatorial optical section of this larva shows 13 cells in the principal ring.

## VI.

### OBSERVATIONS ON FRAGMENTS OF THE FERTILIZED EGGS AND ON THE ISOLATED LOBE.

Extremely interesting and curious results are obtained by a comparison of the behavior of fragments of *fertilized* eggs, and of the isolated polar lobe, with that of fragments of the unfertilized eggs described above.

(a) *The behavior of fragments of fertilized eggs obtained before cleavage.*—In order to make sure that the eggs were fertilized the operation was delayed until one or both polar bodies had been formed, and the egg was then cut as nearly as possible horizontally, so as to separate the lower polar area from the nucleated part. As already described, if this operation be performed on the unfertilized egg, and the two fragments be fertilized, both may, and frequently do, develop. When, however,

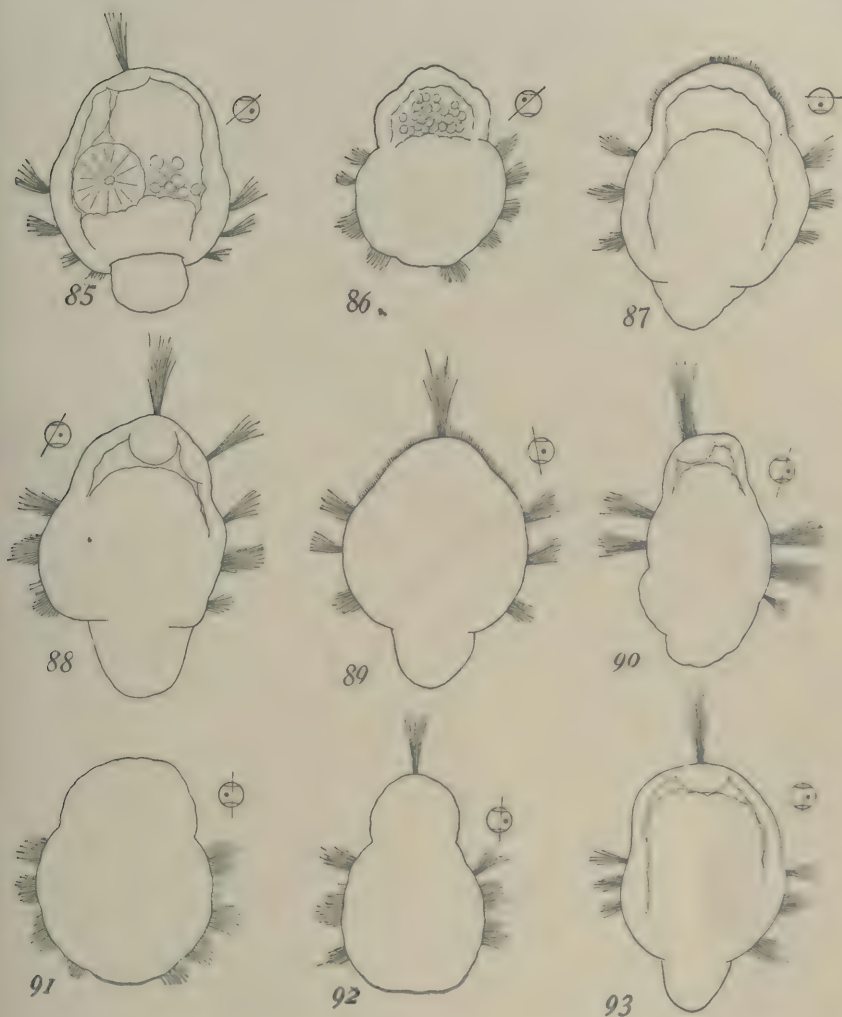


FIG. XI.

*Larvae of 24 Hours, from Egg-fragments.*

85, 86, Twin larva of 24 hours, oblique section passing outside lower polar area, 85, the lower, 86, the upper larva; 87, larva from lower two-thirds, horizontal section, without apical organ; 88, larva from lower two-thirds, oblique section, two apical organs; 89, larva from nearly vertical section; 90, larva from smaller fragment, slightly oblique section bisecting lower area; 91, 92, twin larvae, produced from 81 and 82 respectively, vertical section; 93, larva from exactly vertical section.

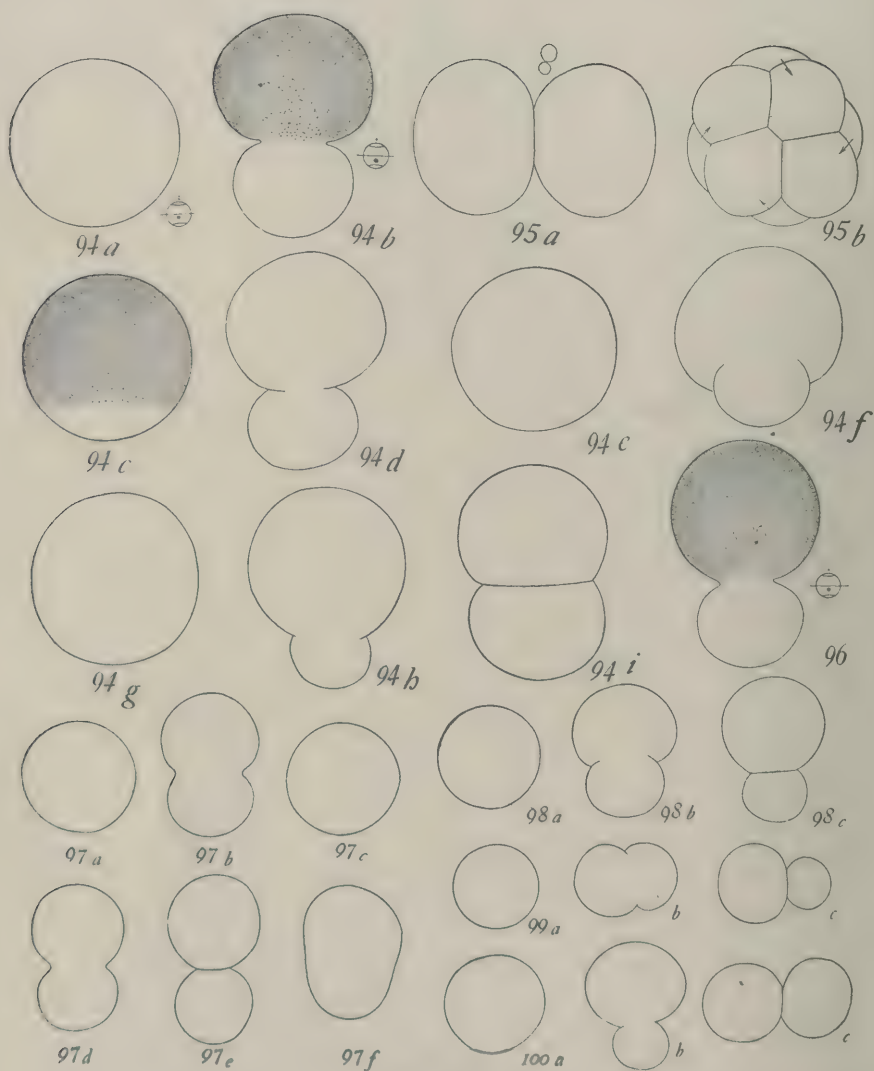


FIG. XII.



*Fragments of fertilized Eggs, horizontal Section; isolated Polar Lobes and  
Fragments of Lobes.*

94, 95, Equal twins from the same egg; 95a, 95b, upper half, 2- and 8-cell stages; 94a-94i, successive changes in the lower enucleated fragment; 94a, soon after operation; 94b, first polar lobe (drawn immediately before 95a); 94c, first resting stage, upper fragment in 2-cell stage (23 m. after b); 94d, second polar lobe (21 m. after c, the upper fragment just divided into 4); 94e, second rest (16 m. after d); 94f, third polar lobe (16m. after last, at nearly the same time with 95b); 94g, third rest (7 m. after last); 94h, fourth lobe (46 m after last, fourth cleavage in progress in upper fragment); after 16 minutes the fragment appeared to be divided into two and so remained; 94i, the same four hours later; 96, lower fragment, like last, but showing correct proportions of first polar lobe; 97, successive changes in isolated polar lobe from the individual shown in Fig. 21; 97a, soon after removal; 97b, first active period (16 m., the egg just divided into 4); 97c, ensuing first resting period (14m. after b); in the second period of activity (not sketched), 15 m. later, as the eggs divided into 8, the lobe constricted as in b, but not so deeply, and again became spherical in a second resting period; 97d, e, third period of activity, 74 and 78 m. after the first period; 97f, final result, 36 m. later; 98, another isolated lobe, 98a, third resting period; 98b, second lobe; 98c, final result, in which condition it remained without further change; 99, 100, two fragments obtained by cutting a polar lobe in two (the original lobe was slightly larger than usual), showing active changes shortly after division of the egg into 4; 99a, 100a, are shown 42 m. after 99 and 100; 99b, 100b, 8 m. later; 99 remained in this condition, while 100 again became spherical by fusion of the two halves and underwent no further change.

a fertilized egg is thus sectioned only the nucleated (*i. e.*, the upper) fragment develops—a result that agrees with my observations on the nemertine egg and that of *Renilla* ('03), and with the earlier ones of Delage ('01) on those of echinoderms. This fragment has essentially the same mode of development as a corresponding fragment of an unfertilized egg, segmenting equally into two and four without the formation of a polar lobe, forming successive symmetrical quartets of micromeres by alternating spiral cleavages (Fig. 95), and producing a larva that is either an irregular monster or a pyriform larva closely similar to those arising from the lobeless egg or the AB half. This is what would be expected in view of the preceding results; but the behavior of the non-nucleated lower half is most remarkable in that *it forms three times in succession a polar lobe from the white area at the same time that the nucleated half is dividing, becoming spherical after each period of activity without dividing.* When this was first observed, I believed that I must in some way have confused the fragments with those of unfertilized eggs; but repetitions of the experiment under conditions that precluded all error, gave the same result. A typical case is shown in Fig. 94, from consecutive camera drawings of the same fragment. The first lobe is shown (Fig. 94b) about 15 minutes after the operation, while the nucleated half (Fig. 95) has just divided into equal halves. Twenty-three minutes later the fragment was again perfectly spherical (94c), while the upper fragment was in a resting 2-cell stage. The second lobe (94d) was formed 44 minutes after the first, while the upper fragment was dividing into 4 equal cells, after which the lower fragment again became spherical (94e, 16 minutes later than 94d). The third lobe (94f) was formed 32 minutes after the second, and was considerably smaller than either the first or the second, as in a whole egg; the upper fragment meanwhile divided into eight cells (Fig. 95b). A third period of rest followed (Fig. 94g). Following the fourth cleavage of the upper fragment the lower one passed through a change no less remarkable than the preceding (it is at this period in the normal development that a large part of the lower polar area passes into the first somatoblast). This

change begins with the formation of a fourth lobe, composed of white material, which is at first much smaller than any of the preceding (94h, 46 minutes after 94g). Unlike the preceding lobes this one was not resorbed into the fragment, but was permanent, slowly increasing in size until after two or three hours it was nearly as large as the remaining portions, the fragment now appearing as if divided into two (94i).

This case is fairly typical of several that were followed through the entire cycle of changes, and one or more of the stages were seen in many individuals. The lobes are not always so distinctly formed as in the one figured, and the final stage, though usually like that described, varies considerably in appearance.

(b) *Behavior of the isolated polar lobe.*—Previous to making the observations just described, I had several times observed changes of form in the isolated polar lobes after their removal from the trefoil stage. On reexamining the matter I found that these changes are also periodic, *taking place approximately at the same time as the cleavage in the lobeless nucleated portion.* The activities of the isolated lobe at these periods vary considerably in different individuals. Sometimes the activity is no more than a slight change of form, the spherical lobe becoming slightly pyriform or even almost amoeboid. Frequently, however, *the isolated lobe actually forms a smaller lobe by a process that closely simulates the formation of a polar lobe by a whole egg or an egg-fragment.* In any case, each period of activity is followed by a spherical resting-stage that coincides approximately in time with the resting stages of the segmenting lobeless portion. I regret that I had not time to study this remarkable phenomenon with sufficient care, but give series of sketches illustrating two particular cases. Fig. 97a shows a lobe soon after its removal; 97b, the same, 16 minutes later just after the egg had divided into four; 97c, the ensuing resting stage, 14 minutes after 97b; a second period of activity followed, in which the lobe again constricted, but not so deeply as at 97b, followed by a second spherical stage; 97d and 97e show the third active period, and 97f the final result, after which no further change occurred. In 98 is shown the final active period of a lobe, which resulted in the permanent apparent



division of the lobe into two. Even if the lobe be cut in two after its removal, the fragments likewise pass through alternating periods of activity and rest closely similar to those of the whole lobe, as is shown in Figs. 99, 100 (the original lobe was somewhat larger than in the other cases shown). This proves that the power of a rhythmic change of form involving the temporary formation of lobe-like structures, is not a property of the lobe as a whole, or of the lower polar area, but is inherent in the substance of which it is composed. It would be interesting to compare in this respect the behavior of the isolated lobe, or fragment of a lobe, with fragments from other regions of the fertilized egg. Such fragments would probably also exhibit rhythmic changes, but I hazard the conjecture that their activity would be found to differ in some definite way from that of the lobe-fragments.

The phenomena above described, which deserve further careful study, are of interest both cytologically and embryologically. First, since both the nuclei and the centrosomes are absent, it follows with great probability that even in the cleavage of a whole egg the constriction of the cell that leads to the formation of the polar lobe takes place wholly independently of either these structures or the astral rays, which suggests the possibility that the same may be true of the constrictions that lead to complete cell-division. Second, since the rhythm in the formation of the polar lobes in the enucleated fragment coincides with that shown in the division of the nucleated fragment, it is clear that as far as the lobe-formation is concerned the cytoplasmic division rhythm is quite independent of that of either the centrosome or the chromosomes. This fact may be placed behind the one earlier determined by Boveri ('97), Ziegler ('98) and myself ('01), that the rhythmic activities of the chromosomes and of the centrosomes are likewise independent, or at least separable. But beyond this it is remarkable that the periodic activity in the non-nucleated fragment is not merely of a rhythmic character, *but changes its character at the time of the fourth cleavage* when in the normal development the material of the polar lobe no longer forms a merely temporary structure, but is permanently cut off by a cell-division. We here catch a glimpse, as it were, of a

definite order of events predetermined in a particular cytoplasmic area and wholly independent of the immediate action of nucleus or centrosome. An additional point of great embryological interest is the fact, shown by a comparison of Fig. 6 with Fig. 94, that in these fragments the polar lobe is, at least in some cases, nearly or quite as large absolutely as in one entire egg; whereas in the lower fragment of an unfertilized egg it is typically reduced to the correct proportional volume of the lobe in a whole egg. This is however not invariable, for in some cases, an example of which is shown in Fig. 96, the lobe is reduced to its proper proportional size. I have not accurately studied this matter in a sufficient number of cases to speak very positively; yet I feel confident that the contrast in this respect between the lower fragments from unfertilized and fertilized eggs is a general, though not an invariable rule. The interest of this fact is pointed out in the sequel.

## VII.

### COMMENT.

Without undertaking at this time a complete discussion of the foregoing observations, I may briefly indicate their bearing on the general questions referred to at the beginning.<sup>1</sup> My observations demonstrate conclusively, I think, both the mosaic character of cleavage in these eggs, and the definite prelocalization of some of the most important morphogenic factors in the unsegmented egg. The *Dentalium* egg shows, even before it breaks loose from its attachment in the ovary, and long before even the initial changes of maturation, a visible definite topographical grouping of the cytoplasmic materials. This is proved by the experiments to stand in definite causal relation to the subsequent differentiation of the embryo in such wise that the removal of a particular cytoplasmic area of the unsegmented egg results in definite defects in the resulting embryo that are not restored by regenerative or other regulative processes within the time-limits of the experiment. Since both the egg-fragments and the isolated blastomeres

<sup>1</sup> A more general discussion of the mosaic-theory of development, with a fuller review of the literature, will be given in a following paper.

become perfectly spherical before development proceeds, the resulting defects cannot be due to a failure of regulation traceable to the shape of the fragment, as was formerly assumed by several writers. Neither are they due to insufficient mass; for perfect dwarfs may arise from fragments much smaller than those that show the characteristic defects. Further, these facts, like those earlier determined by Crampton ('96) in the gasteropod egg, and by Driesch and Morgan ('95) and more recently by Fischel ('98) in the ctenophore egg, are fatal to the view that embryonic differentiation is brought about through qualitative nuclear division during the cleavage. The conclusion is therefore unavoidable that the specification of the blastomeres in these eggs is due to their reception, not of a particular kind of chromatin, but of a particular kind of cytoplasm; and that the unsegmented egg contains such different kinds of cytoplasm in a definite topographical arrangement. How many such specific stuffs exist in the unsegmented egg of *Dentalium* and what is their arrangement it is impossible at present to say; for the pigment-band and the two polar areas can only be considered as an outward sign of an organization that for the most part doubtless escapes the eye. My experiments have only positively determined the cytoplasmic prelocalization in the lower polar area of material essential for the development of that complex of structures that I have included in the term "post-trochal region," and of one other structure, the apical organ. The first of these includes material that is essential to the development of the typical larval form, including the foot, to certain characteristic ectoblastic structures of the post-trochal region, such as the shell-gland, mantle-fold, and probably also the pedal ganglia; it also appears probable that it includes material essential for the formation of the cœlomesoblast. I do not doubt that further experiments on this egg will show a still more definite and detailed prelocalization; though, as already stated, it is not easy to determine this, owing to the difficulty of distinguishing between defects in the partial larvae that result directly from the plane of section and those that are due to other causes.

Two additional facts clearly appear from the experiments, on which I would lay stress. First, the amount of material removed



with the polar lobe or lower polar area is wholly disproportionate to the effect produced. The polar lobe includes less than one-fifth the volume of the egg; yet its removal does not merely cause a structural defect of like extent, but inhibits the whole process of growth and differentiation in the post-trochal region and the concomitant withdrawal of the pre-trochal region. The cleavage of the lobeless embryos shows that both the second and the third quartets are formed; and it is fair to conclude that certainly in the AB half of the embryo, and probably also in the CD half, these cells contain ectoblastic material, which in a normal embryo would contribute to the formation of the post-trochal region. These cells, as stated above, close in around the posterior region, and perhaps are partially turned in with the invaginating entoblast-cells. In any case, however, the power of active growth in the post-trochal region, so conspicuous in the normal larva, is wholly lost with the removal of the excess of material in the D quadrant. It does not seem possible that this loss in power of growth is due to mechanical obstacles, since the same defects exist in fragments of the unsegmented egg from which the lower polar area has been removed and which are free to segment as best they can. The conclusion therefore appears unavoidable that the material of the lobe is not only specifically necessary for the formation of the bases of the post-trochal structures, but also for the whole growth-process that is here brought to a focus. Apart from its more general bearings, this conclusion is important from the light that it may throw on the teloblastic growth of annelids and other segmented forms, and it seems altogether probable that if the polar lobe could be removed from such an egg as that of *Sabellaria* or *Myzostoma* the resulting larva would fail to develop a metameric trunk-region.

A second point of interest that clearly appears from the experiments is that the topographical grouping of specific materials in the unsegmented egg may be in its *ensemble* widely different from that of the definitive bases of the organs which they determine; for the experiments demonstrate that the apical organ, lying at the upper pole, is determined by material originally lying far down in the vegetative hemisphere in the lower polar area.

On this point an analogous result has recently been obtained by Yatsu, who has shown with great probability that in the unsegmented nemertine egg the basis of the apical organ does not lie at the upper pole, where we should expect to find it, but in, or slightly above, the equatorial region.

These facts have an important bearing on our interpretation of development in general. In my previous paper on the nemertine egg I have developed an hypothesis of differentiation agreeing broadly with Sach's well-known theory of formative stuffs, and with the general conclusions regarding mosaic development independently published by Fischel ('03) nearly at the same time, the essential assumptions being that the prospective value of a cell is determined by its cystoplasmic content, that this content is determined by the form of cleavage in connection with an antecedent formation and segregation of specifically different materials (which may itself determine the form of cleavage), and that the morphogenic function of cleavage, so to say, is to isolate the materials thus segregated. This conception, it is hardly necessary to point out, receives very definite support by the observations now brought forward; but I wish to bring them more closely into relation with those made on the nemertine and echinoderm eggs, especially with regard to the general question of progressive (*i. e.*, epigenetic) localization in the egg. In the nemertine (*Cerebratulus*) I found that either an isolated blastomere or a fragment from any region of the unsegmented egg may produce a perfect dwarf larva; but the two differ in the form of cleavage, the blastomere segmenting as if still forming part of a whole embryo and producing an open blastula (as in the echinoderm), while the egg-fragment segments like a whole egg and produces a closed blastula—that is, it develops as a whole from the beginning. I explained the contrast in development between the two as the result of a regrouping of the egg-materials, occurring during and subsequent to the process of maturation and fertilization, which initiates the morphogenic process and determines also the form of the earlier cleavages. I pointed out that such regrouping of materials is known to occur at the maturation-period of many eggs—for instance, in the sea-urchin—and suggested

that the contrast between the development of an egg-fragment in the nemertine and in a sea-urchin (where it segments like a whole egg only after section in certain planes) is owing to the fact that in the latter, egg-fragments have only been obtained in the period subsequent to maturation when the regrouping has been effected. Localization of the cleavage-factors was thus conceived, essentially in agreement with Roux's early conclusions regarding the frog's egg, as a progressive (*i. e.*, epigenetic) process, and the same conception was applied to the general morphogenic process which, as is shown with especial clearness by the facts here brought forward, may be so closely connected with the cleavage-process.

As far as the progressive character of localization is concerned, the result obtained in *Dentalium* may seem at first sight to be in disagreement with the conclusions just reviewed, for the germ-regions are here defined by a definite segregation of materials that exists even in the attached ovarian egg long before either maturation or fertilization, and the isolated blastomere is not capable of producing a complete embryo. But the contradiction disappears upon comparison with certain other forms, which are intermediate in character between the extremes represented by *Dentalium* and the nemertine or echinoderm egg; and this comparison demonstrates, as I believe, the validity of the theory of "precocious segregation," formulated as a pure speculation by Ray Lankester in 1877. I have already expressed the opinion that the horizontal stratification of the egg expressed by the three zones of material visible in *Dentalium* or *Myzostoma* is comparable, or at least analogous, to that which finds an expression in the formation of the well-known polar rings of leeches and oligochaetes. This comparison is based both on the position and mode of formation of these rings and on their fate. Vejdovsky ('88) very clearly shows that in *Rhynchelmis* both the polar rings arise as local thickenings of a general ectoplasmic layer, and both assume at one period the form of protoplasmic discs lying at either pole of the egg (as Whitman also observed in *Clepsine*). Except for the fact that the upper and lower protoplasmic areas have not at any period been seen to appear in the form of actual



rings, the resemblance to these relations of those observed in *Dentalium* is unmistakably obvious. It is entirely possible that the correspondence is not complete; but that in a general way the resemblance indicates a similar form of stratification in the molluscan and annelidan egg, seems hardly open to question; and the comparison is sustained by the fact that in *Clepsine* both rings were traced by Whitman into the AB half, and the upper one into the D quadrant, while in *Rhynchelmis* Vejdosky traced both rings into the D quadrant, where the material of the two fuses into one mass in the 4-cell stage and later *passes into the mesomeres*, which are undoubtedly to be identified with the somatoblasts.<sup>1</sup>

If this comparison be admitted a further comparison of these and some other forms is highly significant. In *Dentalium* three structural zones are present from the beginning, the lower one coinciding in extent with the lower white area, the upper one lying at the centre of the upper white area, at first very small, but rapidly increasing in extent during and after the maturation period. A condition similar to this exists in *Sternaspis*, where Vejdosky ('81) showed that a distinct protoplasmic area, *which he compares to a polar ring* ('88, p. 122) lies at each pole of the ovarian egg, the upper one being much smaller than the lower one, though larger than in *Dentalium*. In *Clepsine* and *Rhynchelmis* three structural zones are likewise present, *but these first appear during the maturation period* with the development of the polar rings, like the three zones described by Boveri ('01) in the *Strongylocentrotus* egg. The egg of *Myzostoma* occupies, at least in some respects, an intermediate position. No upper protoplasmic disc has here been observed as yet, but the lower protoplasmic area is obviously represented by the green mass, which, as Driesch ('96) has shown passes into the polar lobe, and subsequently certainly in part into the first somatoblast, and probably in part into the second somatoblast, precisely as in *Dentalium*. The interest of this case, compared with the foregoing, lies in the fact observed by Driesch (which I can confirm) that before ma-

<sup>1</sup> Cf. Vejdosky and Mrazek ('03, p. 454); see also the highly interesting statement (p. 534) that the dense protoplasm of the polar rings ("Polplasmen") can be recognized as such "in den Zellen des Mesoblasts insbesondere in den grossen Mesomeren."

turation the egg shows at first but two colored zones, of which the lower green one exactly represents the lower white area of *Dentalium*, while the upper one first segregates during maturation into an upper red zone and an equatorial colorless one. Like the lower zone the two upper ones correspond very closely in fate to those in *Dentalium*; for the upper (red) area passes into the ectomeres, like the upper white area of *Dentalium*, while the middle (colorless) zone passes into the entomeres, as is the case with the greater part of the middle (pigmented) zone in *Dentalium*. It is possible that sufficiently careful search may reveal the presence in *Myzostoma* of an upper protoplasmic disc, comparable with a polar ring; and as far as the visible colored zones are concerned, it is evident that the *Myzostoma* egg stands midway between those of *Dentalium* and *Strongylocentrotus*, and it is probably intermediate also between *Dentalium* or *Sternaspis* and *Clepsine* or *Rhynchelmis*.

It seems a legitimate interpretation of the foregoing series that these eggs present an essentially similar form of stratification *which is attained at different periods in the ontogeny*, and that as compared with the leech or oligochaete, *Myzostoma* and *Dentalium* or *Sternaspis* represent two earlier stages in the precocious segregation of specific cytoplasmic materials that have a like prospective value in the development.<sup>1</sup> But if this be admitted, it follows that in none of these cases can the segregation in question be considered as a primary character or "preformed quality" of the egg. Upon this secondary localization of material, as my experiments prove, depend many of the most important features of the later morphogenic localization; and I think a presumption is thus established that cytoplasmic prelocalization is in general of like secondary or epigenetic origin, though to what extent this holds true can only be determined by further experiment.

Although the characteristic segregation is in its main outlines effected very early in the egg of *Dentalium*, it may be pointed out that, like so many other eggs, there is the clearest evidence of

<sup>1</sup> Cf. Vejdovsky "Während aber bei *Sternaspis* die Concentration des Bildungsplasma an beiden Polen bereits im Laufe der Eibildung stattfindet, sammelt sich dasselbe bei *Rhynchelmis* erst nach der Polzellenbildung und dem Eindringen des Spermatozoön in das Ei an" ('88, p. 122.)

later movements and progressive segregation of the cytoplasmic materials. I will only call attention, among these, first, to the determination of the apical organ by material originally lying in the lower polar area, which, if my interpretation of the experiments is valid, moves upwards to the apical pole in the period between the first and second cleavages. That such a movement occurs is only a matter of inference; but this interpretation appears to me far simpler and more intelligible than to assume a brief "Fernwirkung," or the like emanating from the first but not the second polar lobe. It is however not a matter of inference but of fact that the remaining material of the lower white area moves upwards and towards one side in the 8-cell stage preceding the fourth cleavage, when it apparently fuses with the material of the upper white area in the D-quadrant. It is interesting to compare this with the facts described by Vejdovsky in *Rhynchelmis*, where the remains of the upper and lower polar rings fuse in the D-quadrant at the 4-cell stage.

I have endeavored to show that cytoplasmic prelocalization in *Dentalium* differs only in degree from the conditions existing in such eggs as those of the nemertine or sea-urchin. The same may be said, I think, of the development of isolated blastomeres, despite the fact that in *Dentalium* such blastomeres are incapable of producing complete dwarf embryos. As in the nemertine or sea-urchin, although the isolated blastomere segments as a part and not as a whole, the embryo finally closes, in the course of which process structures like the prototroch, the post-trochal and pre-trochal regions, and the gut, close to form whole structures. That this process, which in the case of the nemertine I compared to Morgan's "morphallaxis" in regenerating planarians or hydroids, falls short of producing a complete embryo in *Dentalium*, may be due to different causes in different cells. In the AB half or one of the smaller quarters this is obviously due in the main to lack of the specific material of the lower polar area. The failure of the CD half or the D quarter may in part be due to a like cause; but since these embryos contain the materials (those contained in the lower polar area) that are missing in the other cases, their failure may be due to a different cause. The CD half



larvae are sometimes nearly normally formed except for the false proportions of the post-trochal and pre-trochal regions. Their invariable subsequent degeneration into irregular and monstrous forms is not improbably due to the abnormal mechanical conditions created by their mode of development. It seems possible, however, that if these larvae could sustain themselves sufficiently long they might in some cases succeed in attaining a normal condition. They die before attaining this end; and hence succeed no better than the AB halves in the "attempt" to produce a perfect embryo.

One cause of the difference between the isolated blastomeres of the nemertine or sea-urchin and the mollusk thus doubtless lies in a difference in the segregation-pattern such that in the former the specific materials are symmetrically divided between the first two blastomeres, while in *Dentalium* such is not the case. In the former, accordingly, the earlier cleavages are purely quantitative, but in the latter are qualitative as far as the cytoplasm is concerned, and to this extent produce from the first cleavage onward a mosaic-work in entire accordance with Roux's general conception, as I long since indicated in the case of *Nereis* ('94). But beyond this the results especially of Driesch's later studies on the isolated blastomeres of sea-urchins indicate that here, although a definite polarized segregation of material has taken place at the time of the earlier cleavages (directly proved by Boveri's ('01) observations on *Strongylocentrotus*, indirectly by Driesch's ('00) comparison of the development of the upper and lower quartets of the 8-cell stage) this segregation is not only symmetrical with respect to the axis but is also less definite or less complete than in the molluscan egg,—again a difference which finds its natural explanation in the theory of precocious segregation (or differentiation). I should therefore interpret the differences between the isolated blastomeres of the mollusk and those of the sea-urchin or nemertine as due to a difference, on the one hand, in the pattern, on the other hand in the degree, of segregation.

It is hardly necessary to point out that the foregoing conclusions will in large measure reconcile the apparent conflict between the fact of cytoplasmic prelocalization and the continually increasing

evidence that the primary determining factors of development are to be sought in the nuclear organization. The well-known hybridization experiments of Boveri ('92, p. 469) and Driesch ('98) on sea-urchins have shown that the earlier cleavage-factors conform to the maternal type and hence must be predetermined in the egg-cytoplasm; and up to the blastula-stage, at least, the embryos remain of the pure maternal type. But the same experiments demonstrate no less clearly that the nucleus begins to affect the cytoplasmic phenomena at least as early as the late (prismatic) gastrula, and according to Boveri's latest work ('03) as early as the mesenchyme-formation, though the latter point is disputed by Driesch ('03). It therefore appears possible, not to say probable, that every cytoplasmic differentiation, whether manifested earlier or later, has been determined by a process in which the nucleus is directly concerned, and that the regional specifications of the egg-substance are all essentially of secondary origin.

Another question, which has been often discussed, is raised by these observations, namely, as to the relation in the regenerative process between the moulding of the mass as a whole (which falls under the general conception of Roux's "Umordnung der Zellen" or Morgan "morphallaxis") and the specification of the individual cells. Like the facts determined by Fischel ('98) in the ctenophore egg (following the earlier work of Driesch and Morgan) those observed in *Dentalium* bring out with great clearness the independence, in this case, of the two groups of factors by which these are determined. It is a very noteworthy fact that all the partial larvae that lack the lower polar area, whatever their size or mode of origin, tend to assume the same form, and all are alike devoid of further regenerative capacity. The larvae arising from entire eggs after removal of the polar lobe only, the CD half from which the second polar lobe has been removed, the AB half, the A, B or C quarter, or an upper fragment, of any size, of the unsegmented egg—all these typically assume the characteristic pyriform shape with the trochoblasts surrounding the larger posterior end. This form, which results after closure of the embryos and gastrulation, is essentially a prolate

spheroid modified by the presence at one end of the large trochoblasts which have not like the other cells the power of continued multiplication, and it evidently represents a state of equilibrium towards which any segmented mass of the egg tends that is devoid of the lower polar area. Whether the closure of the embryos (which in the case of isolated blastomeres are at first strictly partial structures) to produce this form should be considered as a regulation or regenerative process is largely a question of definition.<sup>1</sup> In any case the facts very clearly show that the process is not perceptibly influenced by the nature of the cells individually considered; nor does it, on the other hand, appear to exert any appreciable effect on the nature of the individual cells ("Umdifferenzierung" of Roux), as will be more clearly shown in my second paper.<sup>2</sup> Certainly the closing of the embryos does not lead to the least perceptible tendency towards the restoration of the missing structures that are dependent on the material of the lower polar area.<sup>3</sup> I am in agreement with the opinion of Fischel ('98) that, whether a regulative process or not, the closing in to form a closed structure is probably explicable as a result of relatively simple physical factors, though I doubt whether the explanation is as simple as Fischel assumes in the case of the ctenophore.<sup>4</sup> It is difficult to avoid the conclusion that these same factors are operative in the establishment of the normal form in a whole embryo; but to them are add in the material of the lower polar area a far more complex group of factors, at present not analyzable, that involve the whole process of growth and metamorphosis. That a mass of cytoplasm so small should

<sup>1</sup> Roux ('93, p. 837) interpreted the closure of the open blastula as part of the regenerative process, in opposition to Driesch ('92, p. 585), who asserted that this had nothing to do with the regenerative process proper; though he afterward took the ground that it should be considered as an initial regulative process ('96, p. 88). Morgan ('01, p. 13, etc.) classes morphallaxis under the head of regeneration, though not the closing in of a cut surface, which is considered as a preliminary process. Cf. Child, on "Mechanical Regulation" ('02).

<sup>2</sup> Cf. Crampton, '97, p. 55.

<sup>3</sup> Cf. the remark of Driesch, based especially on Crampton's experiments on *Ilyanassa*; "Ist, wie bei Gastropoden und Anneliden, echte Lokalisation der Bildungsfaktoren im Ei anzunehmen, so schliesst das eine Regulation zum Ganzen wirklich aus." ('96, p. 89.)

<sup>4</sup> Cf. Rhumbler, '02, Zur Strassen, '03.



exert so great an effect on the morphogenic process is a most convincing piece of evidence in favor of the theory of specific formative stuffs in development. The only intelligible view of the polar lobe seems to me to be that it is, so to say, a reservoir of such stuffs destined for allotment to particular cells which thereby become definitely specified, irrespective of their subsequent relation to the embryo as a whole. This is a very different result from the oft-quoted one of O. Hertwig that the lineage of particular structures from particular blastomeres is nothing more than an incidental result of the continuity of development. It is equally opposed to the conclusions of other writers who have too hastily rejected the principle of mosaic development for which Roux and others have contended.

Lastly I may point out that in so far as these observations show the course of differentiation, and the correlation of parts, to be determined by a preëxisting topographical grouping of specific egg-materials they sustain an essentially mechanistic (as opposed to a vitalistic) interpretation of development. To conclude however that these eggs are devoid of regulative capacity would be to overlook some of the most striking of the phenomena I have described. The experiments give clear evidence that a power of regulation exists in the unsegmented egg that is no less striking in form, if more limited in degree, than in the nemertine or echinoderm. As in the case of the nemertine, the typical spiral cleavage, alternately dextrotropic and leiotropic, is not affected by section in any plane. Far more striking is the fact that *in the cleavage of an egg-fragment the size of the polar lobe, on which the proportions of the trochophore largely depend, is proportional to the size of the piece*. Since this is true even after horizontal section, when the whole of the lower polar area is included in the piece, it follows that *the predetermination of this area is qualitative, but not quantitative*, or only quantitative in so far as it is subject to regulative control by other factors. This conclusion receives further support from the one reached above that the material of the lower polar area is as such specifically concerned not merely with the formation of the structures that arise from it but with the form of growth that results in the metamorphosis. But if this par-

ticular area shows such a qualitative, as distinguished from a quantitative, pre-determination, one is led to suspect that a like conclusion may apply to other egg-regions, such as those that form the gut, the prototroch, and the like; and to conclude that however detailed a prelocalization may exist in the form of regional segregations of material, a regulative factor may always be present that controls their normal combination. In this respect the unsegmented egg, may, I believe, be directly compared with such an adult animal as a planarian or hydroid, which, while possessing more or less definitely specified tissues, in a typical grouping, nevertheless may possess a high regulative capacity shown in the process of regeneration after injury.

The facts observed give as little clue to the nature of the regulative factors by which the quantitative relations are determined in the egg-fragment as in the fragment of a planarian or hydroid; but one or two considerations deserve brief mention. It is noteworthy that although the polar lobe regularly forms in the non-nucleated vegetative half of a fertilized egg it is as a rule, though not always, *not reduced*, but nearly or quite as large as in a whole egg, whereas in a fertilized fragment, representing the same region of an unfertilized egg, the lobe is as a rule reduced to its proper proportional volume. While I would not lay too much stress on this without further study, it seems to indicate that the power of regulation, on which the size of the lobe depends, is more complete in a nucleated fragment than in an enucleated one. Second, when once the polar lobe has formed, the power of regulation seems to be lost, at least temporarily; for if a part of it be cut away the second lobe is of correspondingly reduced size, as is also the post-trochal region of the resulting larva. This result is supported by the fact that, like the post-trochal region to which it gives rise, the polar lobe in the first (virtual second) division of the isolated CD half, though sometimes slightly reduced, is in general nearly or quite as large as in a whole embryo. These facts prove that the size of the lobe is not determined merely by the size of the piece, but by more complex conditions existing apparently for only a brief period, and apparently also more effective in a nucleated than in a non-nucleated protoplasmic

mass. This sufficiently indicates the complexity of the problem with which we are dealing, and the importance of further more precise studies of the facts. At the same time, it seems clear that the problem of proportionate development in a fragment of an organism here appears in a much simpler form than in a blastula-fragment, or a piece of an adult organism such as a planarian or a hydra; and I think we should not abandon the hope of finding for it a relatively simple solution. While I am not able to offer such a solution, it seems to me that it would be rash to deny its possibility, not merely in the present instance, but in all analogous processes, even when they take place under the more complex conditions existing in multicellular masses.

## VIII.

### SUMMARY.

1. The *Dentalium* egg shows from the beginning three horizontal zones, an equatorial pigment-zone and two white polar areas. Each of the polar areas includes a specially modified protoplasmic area probably comparable to a polar ring.

2. During cleavage the pigmented zone is allotted mainly to the entomeres, the upper white area to the ectomeres, the lower white area to the first and probably also the second somatoblast. At the first, second and third cleavages the lower white area temporarily passes into the "yolk-lobe" or polar lobe.

3. Removal of the first polar lobe leads to a symmetrical cleavage without the subsequent formation of polar lobes, and to the formation of a larva devoid of post-trochal region and apical organ. Removal of a portion of the first lobe produces a larva with reduced post-trochal region, and with or without apical organ. Removal of the second polar lobe produces a larva without post-trochal region but with an apical organ.

4. The lobeless larvae undergo no metamorphosis, form no foot, shell-gland or shell, no mantle-folds, no pedal ganglia, apparently no mouth, and probably no cœlomesoblast-bands.

5. The isolated AB half or A, B, or C quarter, produces a closed larva closely similar except in size, to the lobeless ones. The isolated CD half or D quarter produces a larva possessing a



post-trochal region as large as in a normal larva, and an apical organ, which dies without undergoing metamorphosis. The CD half from which the second polar lobe is removed produces a larva like that from an AB half, but possesses an apical organ.

6. The isolated micromere 1d produces a mass of ectoblast-cells bearing an apical organ, while 1a, 1b, 1c produce no apical organ.

7. Fertilized fragments of the unsegmented unfertilized egg, obtained by horizontal or oblique section, differ in development according as they do or do not contain the lower white area. The upper fragment segments symmetrically without the formation of polar lobes and produces a larva similar to the lobeless ones. The lower one segments like a whole egg of diminished size, and may produce a normally formed dwarf trochophore.

8. Fragments obtained by vertical section through the lower white area may segment like whole eggs and may produce nearly normally formed dwarf trochophores.

9. Enucleated fragments, containing the lower white area, of fertilized eggs, pass through alternating periods of activity and quiescence corresponding with the division-rhythm of the nucleated half, and form the polar lobes as if still forming part of a complete embryo. The same is true of the isolated polar lobe.

10. The foregoing observations demonstrate the prelocalization of specific cytoplasmic stuffs in the unsegmented egg and their isolation in the early blastomeres. The lower white area contains such stuffs that are essential to the formation of the apical organ and the complex of structures forming the post-trochal region, including the shell-gland and shell, the foot, the mantle-folds and probably the cœlomesoblast. These stuffs are contained in the first polar lobe, but the second lobe no longer contains those necessary for the basis of the apical organ. Progressive changes therefore occur in the original distribution of the specific cytoplasmic materials.

11. Comparison indicates that the conditions observed in the molluscan egg differ only in degree from those in the nemertine or echinoderm. These differences reduce themselves to differ-

ences in the period of segregation (or differentiation) and in its pattern, and are explicable under the general theory of precocious segregation.

12. The early development of egg-fragments indicates that the specification of the cytoplasmic regions is primarily qualitative, but not quantitative, or if quantitative is still subject to a regulative process that lies behind the original topographical grouping of the egg-materials.

13. The development of the molluscan egg is in its essential features a mosaic-work and sustains the theory of "Organbildende Keimbezirke."

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# REGENERATION IN RHIZOSTOMA PULMO.

BY  
CHARLES W. HARGITT.

WITH 6 FIGURES.

## I. INTRODUCTORY.

The several experiments, of which this paper presents a resumé, were conducted during the early summer of 1903, at the Naples Zoölogical Station, while occupying the table of the Smithsonian Institution, for the courtesy of which it is a pleasure to express my obligations.

The primary object of the experiments was to test the regenerative capacity of the Scyphomedusae and to institute certain comparisons between these results and those obtained by similar experiments previously made upon the Hydromedusae. So far as I am aware no similar experiments have been made upon the Scyphomedusae with the definite purpose of testing this particular aspect of their physiological constitution. Romanes in his experiments upon "Primitive Nervous Systems," '85, has recorded incidentally the fact that certain mutilations of medusae are promptly healed, but gave no details. Eimer, '78, has also carried on similar experiments and with the same general purpose of testing the character and distribution of nervous centers, but makes no reference to the matter of regeneration. And quite recently Uexküll, '00, has likewise reviewed these experiments of Romanes and Eimer and carried them somewhat farther than they had done. But while arriving at somewhat different conclusions, drawn from a series of experiments in some features coincident with those to be described now, he makes no reference to any regenerative processes, devoting attention almost exclusively to the movements, specially those of rhythmic character, and seeking physical explanations of them.

The earlier references of Haeckel to the capacity of larvae of certain medusae to regenerate entire organisms are likewise indefinite. Morgan in referring to the subject in his recent book on "Regeneration," '01, merely remarks that among Scyphozoa 'the jelly-fishes belonging to this group have a limited amount of regenerative power.'

I very much regret that an unusual scarcity of material compels me to leave several points somewhat less fully considered than is desirable, but I trust they are not of sufficient gravity to seriously mar the general value of the results as a whole.

In one respect this scarcity of material, making necessary successive experiments on the same specimen in many cases, proved fortunate rather than otherwise, since facts of importance were thus brought to light which might otherwise have been overlooked. Some of these will be referred to specifically in another connection.

## II. EXPERIMENTAL.

The experiments were performed upon *Rhizostoma pulmo*, one of the most common of the Mediterranean medusae. Both in size and vigor this medusae affords one of the most satisfactory forms for experimentation which has come under my observation. It seems likewise to suffer less under the somewhat artificial conditions of the aquarium than any other which I have had occasion to use. As compared with *Aurelia* and *Cyanea* of New England waters it is incomparably superior in every way, but particularly in its ability to thrive for weeks in an environment which would prove fatal to the others in as many days. With the single exception of *Gonionemus* I know of no other medusa which affords so good a type for this sort of observation and experimentation. It was not unusual to have specimens under direct observation in the ordinary aquaria of the laboratory rooms for from four to six weeks and without apparent deterioration, even in some cases under the severe tax of extensive mutilation made necessary by the experiments to which they were subjected. It should be stated however that as a rule younger and smaller specimens proved much better than those of larger size; the latter, on account of



their greater mass, are inclined in most cases to sink toward the bottom of the tanks, where after a time certain disorganizing influences appeared to set up pathologic conditions which seemed to deplete their vigor and at the same time render their regenerative processes less satisfactory.

The experiments were directed to three ends, namely to determine: 1, The capacity of the medusae to reproduce lost parts, or to recover from such injuries as might ordinarily happen to them in a state of nature, such as the battering effects of waves, the injuries inflicted by enemies, etc.

2, The comparative powers of the various regions to regenerate, or in other words, the relation of the regenerative capacity to liability to injury.

3, The capacity to regenerate such highly specialized organs as rhopalia, or other sensory structures.

The experiments included specimens of sizes from about 20 m/m to 125 m/m in diameter, and while all proved to have unexpected powers of regeneration those of medium size, from 40 to 70 m/m, proved very much more satisfactory than those of larger size both in convenience and in their promptness in responding to the several sorts of operations, and they apparently were more healthy and vigorous during the progress of the experiments than were those of larger size. Those having a size of 100 m/m or more in diameter proved to be much less prompt in regeneration and, as will be seen in the records of experiments, were much more liable to deteriorate or utterly collapse than were the smaller specimens. This is only what might be more or less expected, and is quite in keeping with observations on other classes of organisms. The same tendency was more or less evident in specimens on exhibition in the public aquarium in which of course no mutilations or similar injuries had occurred. In this connection may be noted a somewhat anomalous pathological phenomenon observed in large specimens both in the exhibition aquaria and in the small aquaria during the course of experimentation, namely, the appearance of whitish blotches, or patches of disintegrating tissues at various places on the exumbrella of the animal which sooner or later affected its health and general behavior.

The matter will be referred to in further detail in another connection and some reference made as to its probable significance and cause.

In all cases the primary experiments were made as soon as possible after the medusae were brought into the laboratory. I have said the *primary* experiments. This refers to the fact already alluded to, that in several cases experiments were variously repeated upon the same specimen. This was in part for the purpose of testing the conclusiveness of preceding experiments, and in part owing to the fact that there was an insufficient supply of material to serve the demands of the course of experiments under way. Details as to these aspects will be given in connection with the several experiments described.

The first experiment was made upon a large specimen, and in order to determine at the outset whether the earlier observations of Romanes and others, that complete removal of the marginal sense organs resulted in complete paralysis of the medusa, these organs were carefully removed by means of triangular incisions as indicated in Figure 1, *a*. The results were substantially confirmatory of the earlier records, the medusa becoming more or less passive, except for an occasional single contraction at very irregular intervals. This experiment was made on May 11, and the following series of observations will suffice to show the general course of events. It should be added in this connection that along with the excision of the rhopalia several other marginal excisions were made, and that three of the oral arms were cut off close below the region of the gastric enlargement. The aspect of the specimen on the next day was practically the same. While there was an occasional contraction of the bell accompanied by certain movements of the body, there were no indications of rhythm.

May 13th.—The medusa, while apparently in perfect health and vigor of general functions, was still unable to originate any definitely rhythmic movements, though responding to various mechanical stimuli, such as a strong current of water from the tap, or the touch of a glass rod. At various times during the day there was evident a rather marked tendency toward spontaneous movements, and occasionally something very like a rhythm,

several contractions following each other in regular succession, though never continuing beyond three or four pulsations.

May 14th.—The medusa, while still more or less passive as before, was yet apparently recovering more of the power of spontaneity, several pulsations occurring at more frequent intervals, but these were not of sufficient vigor to produce any locomotion.

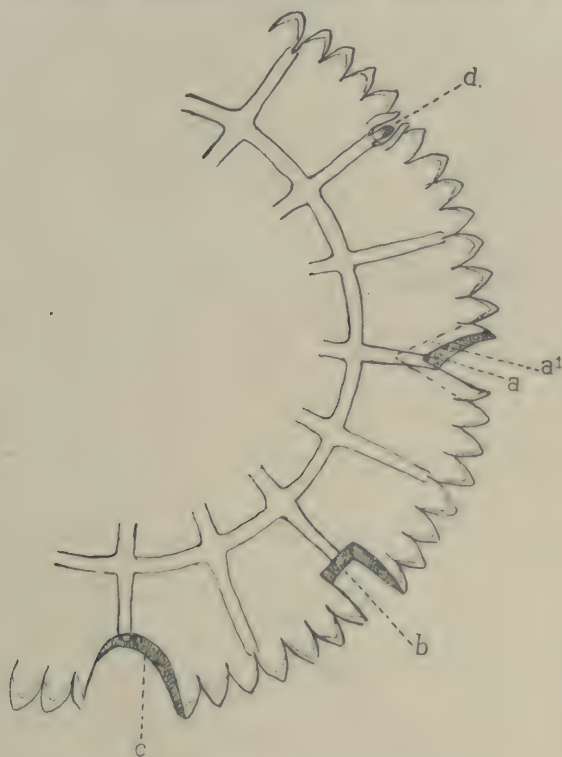


FIG. 1.

*Diagram showing methods of excising rhopalia.*

*a*, usual triangular excision; *a*<sup>1</sup>, excision of larger mass; *b*, rectangular form of excision; *c*, circular form of excision; *d*, form of rhopalium and lappets.

May 15th.—During this and the following day there was an apparent relapse of the medusa to the condition of the first day. There was also less vigor apparent, such stimuli as those referred to above producing but slight effects. This condition continued during the 18th, 19th and 20th.



May 21st.—The medusa seemed to have recovered the vigor or tone to which reference has been made above. There was also a very evident rhythm in the contractions, often as many as ten or more regularly recurring pulsations occurring at irregular intervals during the day. As before, however, they were not of sufficient force to secure the locomotion of the animal. The same condition was observable during the following day.

May 23d.—There was again a marked decline in both vigor and general tone of the body, which showed evident signs of degeneration. This condition continued during the following day, and on the morning of the 25th the medusa was found to have died during the preceding night.

Upon careful examination it was found that wherever tissue had been mutilated or excised there had been a definite healing of the wounds and in the case of the oral arms there were indications of new growth. I was not able to distinguish that there had been any regeneration of the sensory organs, and this will appear somewhat surprising in the light of the following experiments. Whether there had really been no regeneration at all, or that I had overlooked the new organs, or whether they may have disintegrated during the night following the death of the medusa I am unable to say. Certain it is, however, that if regeneration had gone forward as markedly as in the following cases one could hardly have failed to distinguish it. I am inclined to believe that the paralysis following the total removal of these organs may have served to delay or inhibit active regeneration.

The next series of experiments differed materially from the former, particularly in that care was taken to retain certain of the rhopalia in order to insure continued activity of the organisms during the progress of the experiment. The number of rhopalia retained varied from one to eight, the latter case serving as a means of testing the relative influence of these bodies on the behavior of the animals and the rate of regeneration.

On May 12th several specimens, averaging only about half the size of the preceding, namely, about 50 m/m in diameter, were experimented upon. In the first one all the rhopalia were retained, but marginal notches were made of varying sizes between the

sensory bodies, and several of the oral arms were excised. In other specimens a varying number of the rhopalia were excised, and in one case all the oral arms were cut off close to the gastric enlargement and on one side including a portion of this organ itself.

I shall not undertake to transcribe in detail the records of each day, but give rather summaries of results as briefly as is compatible with clearness, trusting that nothing of importance may be sacrificed in the attempt to bring the records within as brief compass as possible.

One of the first effects distinguishable in these and following experiments was the evident quickening of the pulsations of the medusae by the process of excision of the organs, or similar operation. Not only was the rate of the rhythm greatly increased, passing from about seventy pulsations per minute as an average for medusae of this size, to ninety, or even one hundred per minute. And this rate continued during the entire day, or at every observation, which was quite frequent, and well on into the second day, when the rate fell to ninety and later to eighty; but it was not till the third day that the rate had fallen to the normal of seventy per minute. An examination at this time showed an evident healing of the wounds and some signs of regeneration. Had this been restricted to the sensory bodies it might have been interpreted as signifying some important relation of these organs to rhythmic activity, but the fact that similar effects were produced upon specimens which had not been deprived of their rhopalia would sufficiently negative such an inference.

Eimer, '74, had noted such an effect following a division of medusae, particularly those which had been divided into halves or fourths, and had undertaken to show that it was chiefly an expression of the reduced size of the organism due to its division, citing the normal rhythm of specimens of varying size as strongly suggesting such an inference.

Romanes, '85, however, was not able to confirm Eimer's contention either in reference to matter of fact or the cause assigned. Romanes, while citing the variation as to the rate of rhythm in

specimens of similar size, is inclined to emphasize what he terms the prepotent influence of certain of the lithocysts (rhopalia) in coördinating the rate of movement, and the presence or absence of such prepotent organs in the portions of medusae under examination.

Forbes, '48, had long previously called attention to the fact of these quickened movements under the influence of various stimuli, citing particularly a result of an experiment which he had made of a similar character to those which I have cited above. In an experiment in which he had, as he expresses it, "paralyzed one half of the animal" by cutting out the rhopalia from one side, he finds "that the other half contracted as usual, though with more rapidity, as if the animal were alarmed or suffering." He remarks farther that "all medusae when irritated become much more rapid in their movements and contract or expand their disks or bodies in a hurried and irregular manner, as if endeavoring to escape from their persecutors." (*Naked Eyed Medusae*, p. 3.)

While in certain details the conclusions of Forbes may be questioned, of his general observations as to matters of fact there can hardly be doubt. Furthermore, whether the suggestions of either Eimer or Romanes are more than approximate guesses, the later observations of Uexküll have rendered doubtful. So far as my own experiments have gone they hardly touch the problem of the cause of such reactions. We may safely conclude that, in any case, they are of the nature of responses to any continued physical stimulus, such as the experiments under consideration certainly were. With the healing of the wounds there would of course ensue a decline of the irritation, which in turn would be followed by a return to the normal rate of rhythm.

On May 26th, or two weeks following the operation, the medusae had measurably regenerated all the excised organs. The notches cut in the umbrella margins had grown out to complete the normal symmetry and there had been developed in the areas the characteristic purple pigment, differing from the color of the uninjured portions only in its intensity. The new rhopalia were apparently normal in everything save size and pigmentation.

It is rather noteworthy that in these experiments certain of the



organs which among the Hydromedusae are most promptly regenerated are here among the most slow to develop; such, for example, as the oral arms and gastric lobes. The fact that in the rhizostomous medusae these organs have no very active function in the capture of food might apparently afford some plausible reason for this difference in the rate of regeneration. In *Goniomemus* the gastric and oral organs are among the most prompt in regeneration, and are, of course, also among the most important in the functional activities of the animal. That this, rather than liability to injury, should be a predisposing factor in regeneration would seem to be confirmed in the case of *Rhizostoma*, for as will appear in later experiments there seems to be no good reason to suppose that the liability to injury, to which these organs are constantly exposed, has anything to do with the capacity for rapid or perfect regeneration.

Additional experiments were begun on May 28th and 30th. In this series the specimens varied in size from 20 to 60 m/m in diameter. As remarked above there was in these cases the same degree of promptness in the responses, which was markedly in contrast with that shown by specimens of considerably larger size, but in the present cases there was also apparent a somewhat less favorable response in the very small specimens. This fact considered in connection with the difficulty of operating easily upon small specimens, emphasizes the value of animals of medium size for such experiments. This conclusion was emphasized throughout the entire course of experimentation.

In part of the specimens of this series only three rhopalia were excised, in others four, in others five. In some the rhopalia were all removed from one side, while in others only alternate organs were removed. In some specimens the same order was observed as to excision of mouth arms and other similar operations. One of the specimens of the series had only one full-sized mouth arm, while the others were in what seemed to be various stages of regeneration. As is well known these organs among medusae of this type are among the most open to accident from attack of fishes or other predatory enemy. The specimen under consideration would seem to confirm the results of these experiments that

these organs are readily regenerated, and that in a state of nature as well as under the artificial conditions of the laboratory. An examination made with the hand lens on June 2d, or only four or five days following the operation, showed the first indication of regenerating rhopalia. As the organ first makes its appearance it is a very minute papilla-like body, and in these cases at the inner, or upper edge of the notch made by the incision. Examined under the compound microscope the papilla appears as a minute, solid bud growing out from the terminal region of the radial canal, though it does not at first seem to be a direct outgrowth of that organ. Very soon, however, there is established a direct connection with the canal, and it is quite easy to distinguish the circulation of the gastric fluid in the little bud, which becomes definitely vesicular, as shown in Figure 2. The growth of the organ, after its vesicular stage is established, is quite rapid and there can soon be distinguished the thickening of the terminal portion to form the lithocysts. Coincident with this stage of development there is discernible the development of the new hood and lappets, accessory organs, and as will be shown in connection with a study of the histology of these organs, the corresponding development of the so-called olfactory and ocellar pits.

In connection with the present series the following experiments were made with a view to demonstrate that, not only in form but in function, the new rhopalia were perfect organs. From one of the specimens just described in which three rhopalia had been originally excised the other five were excised on June 5th, or seven days after the original experiment. If the three regenerated organs had not yet attained to functional utility the effect of removing the others would, of course, result in the typical paralysis, as in the first experiment already described. As was anticipated, the careful removal of all the rhopalia except the three regenerated ones did not in the least interrupt the normal rhythm or activity of the creature, save to act as a stimulus to quicken it, as already cited in connection with a previous series. This experiment was repeated upon several others of this as well as subsequent series, and always with the same results, except in a single case which may as well be cited in this connection, though coming under later experiments.

In this case the original operation had removed six rhopalia, leaving but two. Soon after the appearance of the new rhopalia, but before they had begun to approach complete development, or before there was any indication of the presence of lithocysts or pigment, the two original organs were carefully removed, and in this case with what might likewise have been anticipated, namely, the complete inhibition of the normal rhythm and the consequent paralysis of the organism. This inhibition continued during the

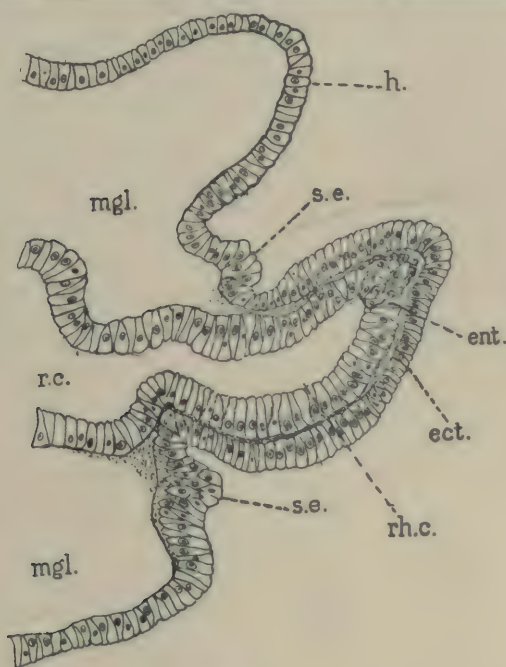


FIG. 2.

Section of rhopalium in early stage of regeneration. ect, ectoderm; ent, entoderm; h, hood; mgl, mesogloea; r. c., radial canal; s. e., sensory epithelium.

following two days. With the continued development of the new rhopalia activity was recovered, though, owing to the interposition just at this juncture of an unhealthy condition of the medusa, it failed to entirely recover the usual vigor or tone which the others had shown.

These experiments, abundantly corroborated by subsequent ones, leave no shadow of doubt, it seems to me, as to the capacity of



these organisms to regenerate in the last detail one of the most highly specialized organs known among Coelenterata. This will be shown more fully in connection with the later account of the histology of the regenerated organs.

Other series of experiments, continued to June 20, while varied in some aspects of detail, were of substantially the same character and with results quite similar to the preceding.

In several of the experiments care was taken to so modify the form and extent of the excised portions as to secure evidence as to the influence of contiguous tissues or parts upon the regenerating organs. In Figure 1 is shown, for example, several aspects of the mode of excising the rhopalia. For the most part the excision was in the form of a triangular cut from the margin inward toward the radial canal, as shown in the figure. The dotted line  $a^1$  will show also in the same connection the occasional extension of the cut to include twice the usual mass. In Figure 1, *b*, will be seen another form of operation. In this case the portion cut out was

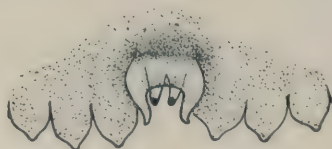


FIG. 3.

Twin rhopalia regenerated in place of the single original one.

rectangular instead of triangular, as in the former. The mass excised in the operation also varied as before. At *c*, in the same diagram, may be seen another form of excision in which the cut was circular instead of angular, as in the former cases. It is interesting to note that, so far as I was able to determine, the form of the excision had no perceptible effect upon the form or rate of regeneration. In the case of the rectangular or circular excisions the new organ appeared in its typical place at the median position of the upper portion of the notch. In the case of the large or small portions excised in the triangular cuts not the slightest difference could be distinguished. With the exceptions of some two or three cases to be considered, there was not the

slightest evidence of any deviation from the exact position occupied by the original organ.

The apparent exceptions referred to are as follows: First, that in at least two cases twin rhopalia were developed instead of the single original one which had been excised. This is well shown in Figure 3. Second, that in one case two rhopalia were regenerated instead of the one originally excised, but unlike the preceding, they appeared at different points — one in the usual position at the upper angle of the notch, the other at the lower, or marginal portion of the notch, as shown in Figure 4.

The mere fact of the occurrence of double rhopalia during regeneration instead of single ones is not of itself particularly remarkable, for the occurrence of such features is not an unusual one in a state of nature, both ephyrae and adult medusae being occasionally found with such double organs. Some further inquiry should, however, be directed to the peculiar position in which the organ noted in Figure 4, at *a*, occurs, namely, at one side of the notch and near the margin instead of the usual position. On the assumption that these organs are of sensory function and correlated with marginal nerve centers it might be

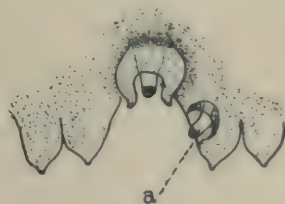


FIG. 4.

Two regenerated rhopalia; *a*, near the margin.

thought that in regeneration they would be likely to occur in close relation with such centers, and that the case under consideration might be thus explained. The fact is very clear, however, that such is not the case with the vast majority of the experiments where apparently the relation of nerve centers had nothing whatever to do with their position in regeneration. And when furthermore we reflect that these are not nervous organs in any true sense, either in their origin or development, though possibly correlated with

some sensory function, it must be more or less evident that such an explanation of the single case cited would hardly hold.

Nor would it perhaps be more satisfactory to appeal to what has been designated as polarity in explaining either series. The occurrence of the organs in conjunction with the radial canals and their apparent differentiation from terminal portions of these structures would seem to afford a much more probable explanation of their regeneration at these apparently predetermined positions. And may we not find in this view a simple explanation of the occurrence of the anomalous case referred to in Figure 4, *a*, for we find near the margins a more or less complex network of anastomosing canals, the presence of one of which may have been the inciting cause of the development of a sensory body at this particular point.

It is interesting to note in this connection that no appearance of heteromorphism occurred during the entire series of experiments. This feature I have referred to in a previous paper, '97, in connection with similar work on *Hydromedusae*. On the assumption that these organs are metamorphosed tentacles we might naturally look for heteromorphic phenomena similar to that recorded among the crustacea, in which occasionally instead of an eye an antenna develops. Nothing of the sort, however, occurred. There seems in every organ and tissue a remarkably inflexible physiological constancy. This is the more remarkable when contrasted with the highly flexible character of the polyp phase of the group among which are found the widest range and variety of heteromorphism.

The fact is not overlooked that *Rhizostoma* is devoid of tentacles, which might be assumed as sufficient reason why heteromorphism of this sort was not manifested. The fact remains, however, that its polyp has the typical tentacular equipment, and that in its metamorphosis they are resorbed and possibly take the usual course, some of them contributing toward the formation of rhopalia. It might be an interesting problem to determine in detail just the extent of this supposed metamorphosis of the polypal tentacles into rhopalia. May it not be possible that the supposed metamorphosis is in reality a resorption and that only,



and that the rhopalia are essentially independent developments such as are found during the process of regeneration? I merely raise the suggestion as it has been forced upon my attention in course of these experiments. It seems worth farther investigation.

In this connection may be briefly described a phenomenon which only came under critical observation late in the course of the experiments, and which for lack of material it was impossible to follow out to conclusive results. Among the last of the series two large specimens were operated upon as follows: In the first all but one of the rhopalia were excised, while in the second all but two were removed. In both cases there was distinctly noticeable an aberrant, rotary sort of swimming movement, the animal revolving in an irregular circle, instead of directly forward or upward as is usual. Examination showed that this inclination of the body in swimming was constantly in the direction of the remaining rhopalia, which would seem to suggest that perhaps they functioned something after the nature of equilibrium organs. I do not recall that this feature has been referred to by the investigators previously cited, and very much regret that it was not practicable for me to carry out such additional experiments as would have afforded more definite conclusions. It must suffice to merely mention the matter, hoping that at some time someone may be able to secure definite conclusions by extended experiments not only upon this medusa but perhaps on others as well.

### III. ABNORMALITIES.

In connection with observations upon several specimens which had become degenerate or perhaps pathologic, resulting from unfavorable conditions of some of the aquaria, or perhaps in some cases due to the depleting effects of the experiments, as in the case of the first experiment cited in this paper, occasion was taken to examine somewhat in detail the observations and experiments of Uexküll and to compare cases coming under my own observations during the course of the experiments.

In one specimen which had shown evident decline of vigor and upon which there appeared certain exumbrellar blotches or cor-

rosion patches, similar to those mentioned in the earlier portion of this paper and comparable in general aspects to cases mentioned by Uexküll, it was found that after all the rhopalia had been removed the specimen yet exhibited certain convulsive contractions which at times simulated an irregular rhythm. I therefore undertook to repeat several of this observer's experiments as to the effects of certain chemical stimuli, specially that of common salt, NaCl. Small crystals of this salt were carefully placed on definite parts of the sub-umbrellar musculature, and I was able thereby to confirm in the main his results. There was a very evident white coloration of the adjacent tissues, and this was followed by a more or less definite, though somewhat irregular, rhythmic contraction of the umbrella which continued for perhaps five minutes. The experiment was repeated several times and upon different specimens and with usually similar results, though differing as to vigor or continuity.

Uexküll had concluded that the recovery of a similar rhythm in specimens upon which he had experimented by excising the rhopalia was due, not to any direct restoration of nervous or other normal equilibrium, but to certain pathologic conditions which had intruded themselves, and among which he was specially impressed by these corrosion abscesses or disease patches, to which reference has been made. Doubting whether an agent of this sort, affecting particularly the exumbrella, could have any very definite importance as a center of stimulus, it occurred to me to vary the experiment by applying the salt to the exumbrellar region instead of the musculature of the sub-umbrella, and though variously repeated the results were uniformly negative in character, no conclusive responses of any sort being obtained. Nor was there observed any of the whitening effects which were so evident in the previous experiments. We may conclude, it seems to me, that the effects produced by the salt in arousing a simulated rhythm of contraction was due to the direct action of the substance on the musculature itself, and not to any general effect produced upon the coördinating centers of the medusa. These stimulating effects of sodium chloride upon muscular tissue are too well known to call for any special mention in this connection.

It would seem, therefore, that in the light of these facts one may well question the validity of Uexküll's conclusions, or rather inferences. The mere presence of whitish blotches on an organism would hardly justify, without the most conclusive demonstration, the inference that the presence of similar effects produced by some reagent proved them identical or even analogous. That there may have been certain pathologic conditions operating upon these medusae of which the whitish blotches were in some respects expressions may have some measure of probability. But that these blotches were in themselves the inciting stimuli giving rise to the simulated rhythm must be regarded as doubtful, if not indeed, highly improbable. Such a conclusion could hardly have been suggested had it been observed that the same whitish blotches are not unusual on specimens which have been for some time in aquaria. Moreover, their presence on such specimens has not in the least, so far as my own observations have gone, served to introduce any variation of the normal rhythm, a condition which might not be unusual on the assumption of these disease patches becoming sources of abnormal stimuli, and thereby introducing erratic or conflicting factors into the physiological processes of the organism. It is well that attention should be directed to disturbing conditions of this character in order that undue weight be not given to a single factor in determining so important a problem. On the other hand it may be quite as important that in discrediting one conclusion there is not substituted another of even less value.

One might be tempted in this connection to go somewhat out of the way to consider Uexküll's conclusions as to the purely mechanical function of the rhopalia in relation to the rhythmic action of the umbrella of medusae. If they might be supposed to act after the fashion of the clapper of a bell, using his figure of comparison, in the case of such medusae as *Rhizostoma* what explanation shall we have for the identical rhythm exhibited by many other medusae entirely devoid of rhopalia or any equivalent organ? Many other objections will immediately arise when one reflects upon the very different histological conditions of structure found in these organs in various medusae, but to take up any one



of these and other phases of the problem would lead too far afield, and we must satisfy ourselves for the time by the reflection that while such speculations are interesting as well as ingenious they are far from demonstrations.

#### IV. HISTOLOGY.

A brief study of the histology of the regenerated organs shows the various stages of the process and establishes beyond doubt a true histogeny, though it has not been possible to demonstrate the details of mitosis in the proliferating cells. This may be due in part to lack of just those refinements of technique necessary to bring out these features. Some of the tissues were fixed by means of Flemming's solution, some by corrosive-acetic acid, and still others in 10 per cent. formol in water. I have not been able to distinguish that there was any appreciable advantage in the one over the others, the formalin seeming to afford equally good fixation and preservation. Heidenhain's iron haematoxylin and an aqueous solution of haematein both afforded fairly good differentiation, though they failed as to the nervous tissues, a result which was not unexpected.

In Figure 2 is shown a longitudinal section of a regenerated rhopalium at a comparatively early stage, when first distinguishable as a minute papilla. In an earlier part of the paper I have referred to its early appearance as having the character of a solid bud from the upper angle of the notch made in the process of excising the organ. From an examination of this figure, which is among the earliest stages I have been able to satisfactorily section, it would seem that in its origin it probably follows the usual process of the regeneration or development of such organs in the coelenterates, namely, that of budding, involving both ectoderm and entoderm. As shown in the figure, there is here a typical outgrowth from the distal end of the radial canal and, as also mentioned in another connection, it was easy to demonstrate at about this stage of development in the living medusa an active circulation in the bud. The cells of the ectoderm at this stage are of approximately uniform size over the entire organ, and the same is also the case with the cells of the entoderm. There

seems also to be present the middle lamella, though less sharply defined than at a somewhat later period. There appears to be a rapid proliferation of the cells of the entoderm near the terminal portion where they form a mass as shown in the figure, though, as mentioned above, it was not possible to distinguish evidence of mitosis.

It is interesting to note at even this early stage the incipient phases in the regeneration of the sensory areas (Fig. 2, *s. e.*) just above and below the rhopalium. The regeneration of the hood is also shown at *h.*

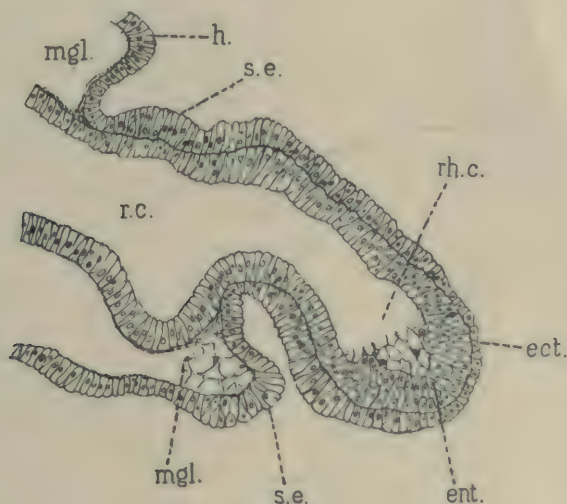


FIG. 5.

Section of regenerating rhopalium. *rh. c.*, rhopalial canal; other letters as in Fig. 2.

In Figure 5 is shown a section taken in the same plane as the former, but at a somewhat later stage of development. The rhopalium has apparently attained nearly full size, but lacking as yet any development of otoliths, though in the network shown at *rh. c.* there is apparently evidence of a differentiation preparatory thereto. There is also shown here the thinning out of the ectoderm of the distal portion of the organ as seen at *ect.*

The sensory areas and epithelium above and below are here seen to have acquired almost their typical form and character as

shown at *s. e.* The hood is also shown at *h*, not having apparently kept pace with the growth of the other organs. Here as before the direct connection of the radial canal with the rhopalium is quite broad and characteristic. The middle lamella, or mesogloea, is shown at *mgl.* above and below, in the latter elements of a loose network being traceable, with the embedded cells, which can also be found indefinitely scattered throughout the jelly.

In Figure 6 we have a section through an almost mature rhopalium, taken in the same plane as the others, only the organ

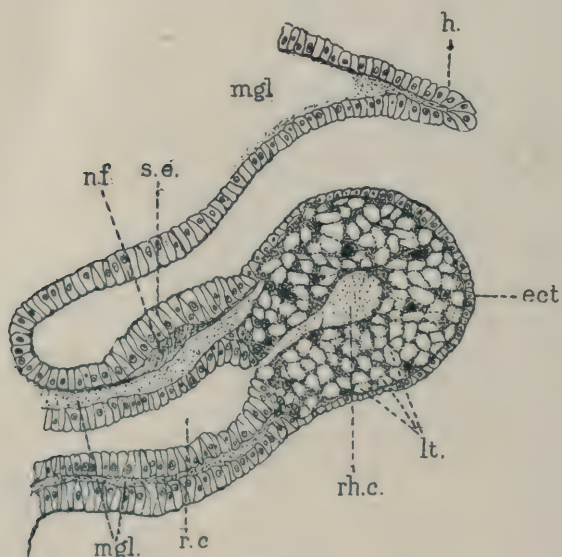


FIG. 6.

Section of regenerated rhopalium, approaching maturity. *lt.*, lithocysts; *nf.*, nerve fibers; other letters as in Figs. 2 and 5.

itself with the terminus of the hood being shown. The ectoderm has become practically uniform over the entire distal portion of the organ, but as it approaches the base of the area of the lithocysts, shown at *lt.* and generally throughout the entire distal part, it becomes columnar. At *s. e.* it forms a definitely arched portion, the sensory epithelium, beneath which at *n. f.* is the so-called nerve fiber area of the nerve center of this region. While it is quite possible to distinguish a more or less fibrous character as



shown in the figure, it has not been possible to trace these fibers into any cellular plexus, or ganglion, such as has been claimed to exist here. Since however my observations in the present instance have been almost entirely restricted to phases of regeneration, it will not be pertinent to discuss the question farther.

As will be seen there is still a continuous connection between the cavity of the distal portion of the organ and the radial canal. This connection Hesse, '95, has shown in figures of normal organs in maturity, but in the present examinations I have found it when fully regenerated to become entirely solid throughout the lithocyst region, the radial canal ending abruptly at its basal end, which is shown almost closed in the figure under consideration.

In the rhopalial cavity, *rh. c.*, which at this time is nearly spherical, there is present a radiating network of delicate fibers, poorly shown in the figure, which seem to diverge from a point on the lower surface and extend entirely across the cavity apparently attaching to the opposite wall. I should consider these fibers of the same nature as those shown in Figure 5 near the terminus of the line *rh. c.*. Though it has not been possible to critically trace the details of the process it seems entirely probable that the entodermic epithelium of this region becomes gradually differentiated into fibers which form the intricate network within which the lithocysts are later deposited. Within this network may be found during the various stages of development the gradual metamorphosis of this entodermic cell mass, the nuclei of the cells often remaining as permanent elements of the organ. Some of the more prominent of these are shown in the figure, and phases of the metamorphosis may be detected near the narrow slit-like canal, just beyond the terminus of the radial canal.

Within the network may also be traced the deposition of the pigment characteristic of the organ.

Concerning the histology of the regenerated oral and gastric organs it has not seemed essential to make special inquiry, since in what has already been shown in connection with the more highly differentiated tissues of the marginal organs it would seem that no serious doubt can remain as to normal histogenic pro-

cesses probably occurring throughout every regenerating organ in this medusa.

It was pointed out in connection with the description of certain experiments that both in form and in function we have among the Scyphomedusae a regenerative capacity extending to the most highly specialized organs. In the subsequent account of the histology of the regenerated organs it has been shown that the process is a perfectly normal and characteristic one, conforming in apparently every detail to the course of development of the embryonic history of the several organs.

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 January 20, 1904.

STUDIES ON REGULATION. IV.  
SOME EXPERIMENTAL MODIFICATIONS OF  
FORM-REGULATION IN LEPTOPLANA.

BY  
C. M. CHILD.

WITH 53 FIGURES.

INTRODUCTION.

The following observations and experiments on *Leptoplana* constitute a part of a series of investigations of form-regulation undertaken at the Zoölogical Station at Naples in 1902-3 during occupation of a table granted by the Smithsonian Institution. A part of the work on *Cerianthus* has already appeared (Child '03a, '03b, '04a.)

The work was undertaken primarily for the purpose of examining the relations between form-regulation and the mechanical tensions resulting from the creeping and other movements in some of the polyclad turbellaria. For this purpose it was desirable that the direction of movement should be relatively definite in at least some one of the forms studied. Many of the polyclads show little definiteness of direction in their movements under ordinary conditions. This is notably the case in the species of *Stylochus* in which, as might be expected, corresponding form-changes are almost completely absent. *Leptoplana* proved to be the only form readily obtainable in large numbers at Naples which fulfilled these conditions. The creeping movements of this form are relatively definite in direction.

If form in the Turbellaria is in any way dependent upon the mechanical strains to which the tissues are subjected, it is to be expected that in forms with tough, resistant tissues, the changes in shape resulting from changes in mechanical conditions will



be less clearly marked than in those with relatively plastic tissues. As is well known, the tissues of many of the polyclads are extremely tough and resistant. Stylochus, Thysanozoön, and many other forms might be mentioned as examples of this condition. As regards this feature also *Leptoplana* proved to be a favorable form since its tissues are relatively soft and plastic, though much firmer than those of *Planaria*. With this form as a basis it was possible to make some very interesting comparative observations upon various other species, which will be discussed later.

Nearly all the specimens used were collected about the Castel dell' Ovo and were presumably *Leptoplana tremellaris*. Since it is impossible according to Lang ('84, p. 482) to distinguish with certainty the species of *Leptoplana* except by examination of the copulatory organs in serial sections, my material may have included other species—*L. alcinoi* and *L. pallida*. Nearly all the specimens used, however, resembled closely the type of *L. tremellaris* represented in Lang's Figure 1, Tafel III. In no case did individual specimens exhibit characteristic differences in the regulative processes that could be regarded as specific, so the question as to the species is in any event of minor importance for the present purpose.

Specimens and pieces were kept isolated or several together according to the experiment, in Stender dishes of various sizes, covered to exclude dust. The water was changed twice a week or oftener, but the animals proved extremely hardy and capable of living in small dishes even during summer for much longer periods without change of water.

An attempt was made early in the course of my work to determine whether the activity of the animals was affected by light. So far as I could determine, specimens kept in darkness were slightly more active; but the difference was not sufficiently great to exert any marked influence on regulation. Later most of the specimens were kept in darkness except when under examination. All were kept without food.

Extensive series of measurements were necessary in the study of the form changes, and *Leptoplana*, like most of the turbellaria,

is not a favorable form for exact measurement. It was necessary to repeat all measurements several times in order to be certain that they were approximately correct. In all cases the attempt was made to secure the measurements while the animal was in the fully extended condition. In order to accomplish this it was often necessary to stimulate the specimens to movement and then to measure them while moving. A small millimeter scale which could be immersed in the water was used for whole animals and the longer pieces, while the smaller pieces were measured under a low power of the microscope with the aid of an ocular micrometer. In all cases the measurements were reduced to millimeters.

The measurements which were commonly made are as follows: length of whole body, distance from anterior end of head to middle of group of eyes, distance from anterior end of head to anterior end of pharynx, length of pharynx, width of head in region of the eyes — in whole animals this is usually the widest part of the body, but in short pieces undergoing regulative changes of form, the widest part is anterior to the eyes; in such cases measurement both of the widest part and the eye-region was made—and finally the width of the body at the posterior end of the pharynx. In regenerating pieces with new tissue the dimensions of the new tissue and the position of regenerating pharynx and genital ducts, if present, were also carefully determined by measurement. Since a cut surface undergoes marked contraction in *Leptoplana* and the new tissue arising from it consequently occupies only a part of the area of the original cut surface it was necessary in many cases to determine with great care the width of the body just anterior to the cut, the width of the new tissue at its origin, the difference of these measurements representing the degree of contraction of the cut surface. Measurements of pieces without cephalic ganglia are not strictly comparable with those of pieces in which the ganglia are present, since the former rarely extend fully. It will not be necessary in most cases to give all these measurements in detail since the figures, which are drawn from them in almost every case, will show the changes with sufficient clearness.

In all cases where the form of the pieces rendered it necessary figures were drawn in my notes on the basis of the measurements and the living specimen. By this means a record was kept not only of the principal dimensions, but also of any special features, *e. g.* the angle between the axis of the new and old tissues, the curved contours, etc. The actual form-relations and contours are, I think, shown by the figures as exactly as is possible in a case where alteration of individual form is so great. Except where otherwise stated the figures are about seven times the natural size. The various internal organs are represented so far as necessary in a conventional manner.

#### REGULATION, NUTRITION AND USE OF PARTS.

As a preliminary to the descriptive part of the paper a brief discussion of certain phases of the problem in hand is desirable in order to clear the ground.

In no case was the attempt made to feed the pieces employed for experiment. In consequence of the absence of food a marked decrease in size occurred during the course of the experiments. There is no doubt, however, that the results of feeding would be similar to those obtained by Morgan with *Planaria* (Morgan, '00), for specimens were occasionally found among the worms collected which had regenerated after a loss of a part of the body. In these specimens the amount of new tissue formed was much greater than in the pieces kept without food, and there is no reason to believe that in pieces where regeneration is possible growth to the full size may not occur, provided enough material is at hand. As Morgan ('98) has pointed out, the material used in the formation of new tissue in starving pieces is obtained from the substance of the piece itself or from its reserve supplies, and the bulk of the old tissue is reduced to a greater or less extent by the formation of the new tissue. When the pieces are fed the amount of new tissue formed is more or less increased and the old tissues not only do not decrease in size, but may grow larger.

The fact that formation of new tissue may occur, not only once but repeatedly, in pieces which have been for weeks without



food indicates that the stimulus which brings about the regeneration is sufficiently powerful to deprive the old portions of material. There is little doubt that this difference indicates a difference in metabolic activity between the old and the growing regions. If this conclusion be correct it follows that in the presence of nutrition the new parts will grow more rapidly than the old. Moreover, we find that in the absence of food growth of the new tissue may cease long before the amount of tissue removed has been replaced. We must conclude therefore either that the stimulus to regeneration decreases as regeneration proceeds, or that the old portions give up material less rapidly as the process continues (Child, '03d). That there is an actual difference in quality between the new and old tissue is clearly shown by observations which I have made repeatedly, viz., that in regenerating pieces kept without food until death occurs from starvation, infection or other causes, the old parts usually disintegrate before the new. In many cases I have seen the old tissue disintegrate almost completely in pieces of *Leptoplana*, while the new tissue remained alive and apparently healthy for a considerable time afterward. Moreover, the new parts in regenerating specimens show a greater degree of muscular and other functional activity than do the old parts.

For reasons which I hope to state in full at some future time, I believe that this difference is at least in part dependent upon functional conditions, viz., that it concerns the use or activity of the parts. In the earlier stages of regeneration other factors are very probably concerned in greater or less degree. The presence of a cut surface places the cells adjoining it under conditions widely different from those existing before the cut was made. The equilibrium in physical conditions is destroyed by the removal of the part and the absence of pressure from other parts on one side may itself be sufficient to bring about a migration or growth of tissue outward from the cut surface. It is extremely difficult in such cases to determine how much of the "new tissue" is the result of migration and how much of actual proliferation. But if the attempt is made by the animal to use the "new tissue" thus formed in the manner characteristic of

the part which it represents new conditions of pressure and tension arise as well as powerful nervous stimuli which probably affect growth and differentiation directly or indirectly.

When for instance the posterior part of the body of one of these worms is removed the animal continues to move about and "attempts" to carry out the same movements as when the posterior end was present, but in the absence of the parts the movements fail more or less completely of success. In fact observation of these cases leads me to believe that in the absence of the part the attempts to attain the usual result are often more powerful than when it is present. For example, a specimen of *Leptoplana* with the tail removed makes violent attempts to hold to the substratum by the cut posterior end of the body as well as by other parts; a specimen with the lateral lobes of the head removed makes violent but unsuccessful attempts to swim; and finally, to take another case somewhat removed from the present considerations, a fish with the tail or part of it removed uses the remaining stump much more vigorously than would be the case if the whole were present.

It may appear at first glance that these statements involve unwarranted assumptions regarding the psychological activities of forms as low in the scale as the Turbellaria, but I believe such a conclusion is not justified. Conscious recognition of the successful or unsuccessful character of the movement is by no means necessary, but on the other hand it is difficult to understand how these creeping worms could advance in a regular, definite manner if the movement over the substratum or the movement of the parts of the body upon each other did not afford certain characteristic stimuli. The movements of these forms as well as those of higher animals are coördinated, and for coördination some stimulus resulting from the movement seems to be necessary. Removal of a part, *e. g.*, the posterior end by which the animal has been accustomed to attach itself, must bring about a change in the relation of the various stimuli. The animals behave in such cases as if they were moving over surfaces to which their bodies do not adhere readily. They appear to make violent efforts to use the parts which are missing. These changes in behavior are dis-

tinct from any irritation due to a wound, for they continue after the wound has closed and new tissue has appeared. There is no doubt, I think, that a modification of the motor stimuli occurs in the absence of a part important to locomotion.

The outgrowth of new tissue from the cut surface is probably, in its earlier stages, the result of the alteration in local conditions consequent upon the removal of a part. But the position of the new tissue, *i. e.*, its connection with a particular part of the old body determines the conditions to which it is subjected in connection with the functional activities of the old differentiated parts. As differentiation in the new tissue proceeds, motor activity appears and soon the movements of the new part are coördinated more or less completely with those of adjoining old parts. Thus the conditions to which the new part is subjected become similar to those which were present in the part removed. These conditions or some of them are undoubtedly formative factors in many cases. In *Stenostoma* (Child, '02, '03) the development of the tail depends in large degree upon their presence.

Now when the new part first shows characteristic coördinated motor activity it is much smaller than the part removed, yet functionally it supplies the place of the other, though at first very imperfectly. But the smaller the size and the more imperfect the formation of the new part, the greater the activity, *i. e.*, the "attempt" of the animal to use it. Thus the new part is visibly more active than the old and if we admit that the conditions connected with this activity are "formative factors" it is easy to see why in a starving piece the new part continues for a longer or shorter time to increase in size at the expense of the old tissue.

As the new part increases in size and its coördinations become more perfect the degree of motor activity decreases, approaching that of the old parts. The extent of regeneration in starving pieces is probably determined by the relative functional activity of the new and old parts. As long as the more intense metabolism of the new part enables it to deprive the old part of material, so long will it continue to increase in size. It is also probable that the old tissue gives up material less and less readily as the encroachments continue. The final result depends on the conditions of the individual case.



It is possible that the effect of the functional conditions may be in many cases largely mechanical, *i. e.*, that in consequence of the use or attempt at use of a growing part, *e. g.*, a regenerating tail, it is subjected to certain mechanical conditions of tension and pressure and that these mechanical conditions themselves constitute in reality the chief "formative factor," acting either mechanically or as physiological stimuli to growth. In many cases, however, there is no doubt that other internal stimuli bring about growth, but even in such cases mechanical conditions must usually play a certain part in the final arrangement of the material produced. In short there must usually and perhaps always be a mechanical factor of more or less importance in regulative morphogenesis. I think it probable that in the lower animals this mechanical factor is relatively simple but of great importance, while with increasing complexity it becomes more complex and more difficult of analysis, though perhaps not less important.

The alteration in general outline and proportion of pieces, especially of the old portions, called by Morgan morphallaxis, which occurs during regulation in such forms as *Planaria* (Morgan, '00, '01), *Stenostoma* (Child, '02, '03) and *Leptoplana*, I believe to be primarily due to mechanical factors connected with locomotion and acting very probably both in a simple mechanical manner and as stimuli to growth, though there is some reason to believe (Child, '02) that the direct mechanical effect is predominant in many cases. We cannot conclude, however, that all phenomena which have been designated as morphallaxis are due to similar conditions. The changes in form of pieces of the medusa *Gonionemus* for instance (Morgan, '99) cannot be due to the factors which cause the change of form in *Stenostoma* and *Planaria*, but are very probably due to physical conditions in the tissues whose equilibrium is destroyed by a removal of a part, and so may be comparable to the inrolling which occurs in pieces of *Cerianthus* (Child, '04a). In dealing with problems of so great complexity generalizations are safe only so far as the actual facts go. Nothing is gained by referring these diverse phenomena to an inherent capacity in pieces for returning to the original form. Such an explanation leaves us exactly where we started.

If these views are correct it follows that these form-changes, at least in the old parts, and often in the new as well, must occur to a greater extent when regeneration is not quantitatively complete, or must be more evident, since the growth of the parts may mask it to a greater or less extent. This is actually

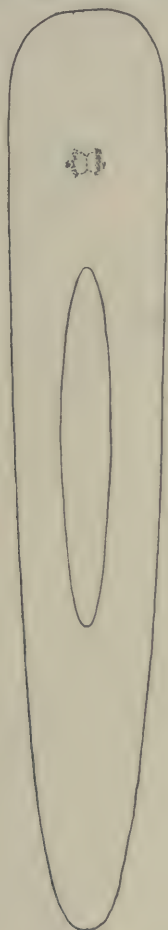


Fig 1.

the case, as Morgan's experiments have shown (Morgan, '00). Since my primary object in investigating the regulative processes in *Leptoplana* was the examination of the alterations in proportion, the most favorable conditions for this purpose, viz., absence of food, were desirable.

On the other hand the study of regeneration in the stricter sense is not at all impossible under those conditions. The failure of the new portion to attain full size is a minor matter. Indeed the presence of food is a complicating factor in the study of regulation of lower forms, since it renders less possible the distinction between ordinary processes of growth and the regulative processes.

#### THE MOVEMENTS OF LEPTOPLANA.

In *Leptoplana*, as in *Stenostoma* (Child, '02, '03a, '03b), there is a close relation between form-regulation and movement. A description of the characteristic methods of movement may properly, therefore, precede the account of experiments.

Locomotion in *Leptoplana tremellaris* is accomplished in two ways, by swimming and by creeping. Lang ('84, pp. 634-636) has described the movements of the polyclads and among them those of *Leptoplana*. I desire, however, to consider these movements with special regard to their mechanical effect upon the tissues and for this purpose Lang's description does not suffice. Figure 1 shows the outline and proportion of a specimen in fully extended condition as when creeping.

Swimming is accomplished by an undulating movement, dorso-ventrally directed, proceeding posteriorly from the anterior end of the lateral regions of the head and anterior portions of the body, the median portions remaining meanwhile almost motionless. This method of swimming is called by Lang the flying movement. In various other polyclads it appears in much more extreme form than in *Leptoplana* and in some involves not only the anterior regions but the whole lateral region of the body as in *Thysanozoön*.

It is interesting to note that in all cases where this undulating movement extends over only a part of the lateral region of the body, the region involved is the broadest portion of the body. In *Leptoplana* it is not sharply marked off from other regions posterior to it, as is the case in some forms, *e. g.*, *Stylochoplana agilis* (Lang, '84, Fig. 2, Tafel II, also pp. 457 and 636). In correspondence with the absence of sharp demarcation of the undulating region in *Leptoplana* we find that the undulating



movements do not cease abruptly as they pass posteriorly but gradually decrease in amplitude until no longer visible. During extreme activity they may extend much further posteriorly than under ordinary conditions and frequently slight undulations of the margins appear along the sides and pass posteriorly even when the animal is creeping. During swimming the anterior region of the body is considerably broader than in Figure 1.

It can scarcely be doubted that these movements play a part in shaping the regions in which they occur. A comparison between frequency, amplitude, and force of the undulating movements and the degree of lateral development in the regions in which they occur is most striking. According to the usual point of view this correlation between structure and function is merely one of the many remarkable cases of adaptation, but in my opinion it is, at least in part, the direct result of function in the individual. Some experimental evidence bearing on this point will be offered elsewhere.

As regards the manner in which the movement may affect the tissues it is not difficult to see that the movement of these parts to and fro through the water must subject them to tension in the lateral direction. This must affect in greater or less degree the distribution and arrangement of the plastic tissues composing the parts. A very simple physical experiment serves to illustrate this point. A cylindrical or square stick of sealing-wax moved to and fro in one plane in water sufficiently warm to soften it will undergo flattening in a plane at right angles to the direction of movement. The change in form is more strikingly shown if a rigid axis is present; a mass of wax molded in cylindrical form about a stiff wire will become in a few minutes a thin, flat plate decreasing in thickness towards the edges and with a rounded outline. The mechanical conditions resulting from the movement of the wax through the water are not widely different from those which the undulating margins of *Leptoplana* produce. If the wire axis of the wax be considered as the longitudinal axis the effect of movement through the water is lateral extension. In *Leptoplana* the undulating movement is confined chiefly to the lateral regions in the anterior third of the body and it follows that the conditions described are limited chiefly to these parts.

There can be little doubt, in my opinion, that these mechanical conditions constitute a factor in the formation of the broad lateral regions in *Leptoplana* and more especially in other forms in which the undulating movements of these parts occur. In other words the form is in some degree the result, not the cause, of the characteristic method of activity. The experimental data to be described support this view.

In addition to its power of swimming, *Leptoplana* is able to creep over surfaces rapidly and in a definite direction. Both muscular and ciliary activity are concerned in the movements, but one or the other may predominate according to conditions.

When the animal is moving quietly, as for instance after a slight stimulation, the cilia afford the chief motive power, although the slight muscular movements of the margins of the body are almost constant, portions being lifted from the substratum, brought forward, and again attached. This muscular play of the margins is especially marked in the anterior regions but extends in some degree along the whole side of the body.

After a strong stimulus the movements take on a different character, becoming chiefly muscular. The portions of the body in which the undulating movements occur during swimming furnish under these conditions the chief motive power. Parts of the margin are lifted slightly, extended in the antero-lateral direction, and attached to the substratum: contraction of the muscles follows and the body is drawn forward. These movements occur in rapid alternation on the two sides of the body and the similarity between this mode of progression and the use of legs cannot escape the observer. The animals appear almost as if walking forward.

At all times during creeping movements the body adheres closely to the substratum as may be demonstrated by sudden attempts to dislodge it. The chief regions of attachment are the lateral margins and the posterior end. Frequently during creeping small portions of the body margin which adhere more closely than other parts are stretched posteriorly to a considerable degree before they are torn away from the substratum. As in many other *Turbellaria* the posterior end is an important organ of

attachment although in *Leptoplana* it is not so exclusively employed for this function as in many other forms.

*Leptoplana* differs from many other species of polyclads in the definite direction of its movements. In some forms, e. g. *Stylochus*, the direction of movement is very indefinite, movements in other directions being almost as frequent as anteriorly directed movements. In *Leptoplana*, however, the deviation from the longitudinal direction is slight.

As a general rule the more posterior portions of the margin and the posterior end itself are used more frequently as organs of attachment than the more anterior regions.

In consequent of the adhesion to the substratum by the margins and posterior end, the body of *Leptoplana* is subjected to mechanical tension in the longitudinal direction, often visibly in a considerable degree, during creeping. As in the case of *Stenostoma* (Child, '02, '03), this longitudinal tension constitutes a factor in determining the general form and outline of the body. The fact that the margins as well as the posterior end are employed as organs of attachment accounts for certain characteristic features in connection with the form.

From the facts above cited regarding movement we must conclude that the posterior portions of the body are subjected more frequently than the anterior parts to longitudinal tension in consequence of their position and more frequent use for attachment, and moreover, that the tension is greater than that in the anterior regions since all ciliary impulses and muscular contractions aiding in forward movement anterior to the point of attachment combine to produce it. This statement is correct in a simple case but frequently various points along the margin may become attached simultaneously and the tension is distributed among them. The continual muscular play of the margins, the rapid transitions which a given region undergoes from attachment to reattachment are of course accompanied by great variation in mechanical conditions. The important point is that the tissues are subjected to longitudinal tension and the posterior regions more than the anterior.



This case differs from that of *Stenostoma* in which the posterior end alone is the chief organ of attachment. Reference to my paper on *Stenostoma* (Child, '02) will show clearly how the characteristic differences of external form between *Stenostoma* and *Leptoplana* may be correlated with the differences in the mechanical conditions to which the tissues are subjected.

My experiments also indicate that the use of the anterior portions of the lateral margins in drawing the body forward constitutes a factor in their development. In consequence of these characteristic, frequently repeated movements these parts are subjected to characteristic physical conditions, which, like the longitudinal tension, must exert some influence upon the arrangement of the cells and tissues.

Anyone who observes the creeping movements of different polyclads cannot fail to note the close correlation between the general outline of the body and the character of the movement. In general the forms which advance in a definite direction are more slender than those like *Stylochus* whose movements are very indefinite in direction. In the last mentioned form lateral movement occurs almost as often as longitudinal, a part of the body-margin being advanced and the other portions drawn up to it by contraction. The breadth of the body is almost as great as the length in *Stylochus*. I am forced to the belief that the forms of the various species are determined in greater or less degree by the conditions of tension, resulting from swimming and creeping movements, to which the tissues are subjected. The experiments to be described afford strong support to this view.

#### THE LIMITS OF REGENERATION.

This section includes merely a brief preliminary statement concerning the power of regeneration in *Leptoplana*. The phenomena will be treated more at length in other connections.

Complete anterior regeneration never occurs in *Leptoplana* when the cephalic ganglia are removed. Removal of all portions of the head anterior to the ganglia and even including the anterior part of the ganglia is followed by rapid and complete regeneration.

When the cut is made at any level posterior to the ganglia neither the ganglia themselves nor the head are regenerated (Cf. Lillie, '01).

Posterior regeneration is qualitatively complete at all levels posterior to the ganglia whether the ganglia are present or absent in the piece, but pieces cut anterior to the ganglia never regenerate the ganglia nor the posterior parts.

Lateral regeneration is qualitatively complete when the ganglia are present, but when they are absent neither they nor the lateral part of the head removed are regenerated though lateral regeneration of other parts may be more or less complete in the absence of the ganglia. Removal of the right or left half of the ganglia is followed by complete regeneration from the remaining half.

In general the amount of tissue regenerated in pieces kept without food is much less than that removed, though all the organs may be present. The amount of posterior regeneration varies inversely as the distance of the cut surface from the anterior end. The size of the piece does not affect the quality of regeneration and affects the amount only slightly, except on approach to the minimal size, when a marked decrease in the amount of regeneration occurs. The minimal size of pieces capable of qualitatively complete posterior regeneration was not determined with exactness, but transverse pieces less than one tenth the length of the body are still capable of qualitatively complete posterior regeneration and pieces even smaller than this, but containing the cephalic ganglia, regenerate completely in all directions.

#### REGENERATION AND MOVEMENT.

Considering first one of the simplest cases, viz., posterior regeneration from a transverse cut surface we find that in *Leptoplana*, as in *Planaria* and other *Turbellaria*, the new tissue which makes its appearance on the cut surface assumes a rounded outline and grows or extends posteriorly in the direction of the longitudinal axis, becoming more slender and tapering as regeneration proceeds. Figures 2—4, drawn from careful measurements, will serve as an illustration of the course of regeneration in such cases.

The cut surface in this piece was a short distance posterior to the cephalic ganglia. Figure 2 represents the piece five days after section, Figure 3 ten days after section, and Figure 4 twenty-seven days after section. The new tissue is bilaterally symmetrical at all times and growth appears to occur most rapidly along the median plane. The gradual decrease in size of the whole is due of course to the absence of food. The course of posterior regeneration in *Planaria*, as described by Morgan and others, is similar,



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.

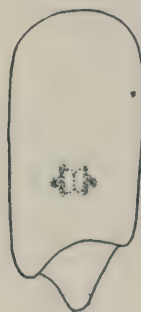


Fig. 6.



Fig. 7.

though the amount of new tissue formed is relatively less than in *Leptoplana*; similar results have also been obtained by others with various forms.

If the cut surface from which regeneration occurs be oblique instead of transverse the course of regeneration differs in some respects from that just described. Figures 5-7 illustrate the history of such a piece, begun on the same day as the pre-



ceding and examined at the same intervals. Figure 5 shows the piece five days after section, Figure 6 ten days after section, and Figure 7 twenty-seven days after section. In Figure 5 the new tissue is symmetrical with respect to the contracted cut surface but not with the median plane of the animal. As growth proceeds however, a gradual change in direction of the axis of the new tissue occurs (Figure 6), until finally this corresponds with the median plane and approximate bilateral symmetry of the whole results, though the new tissue is still unsymmetrical in form since the surface from which it arose is oblique.

This change in the direction of regeneration is also familiar to students of regeneration, having been described by Morgan and others for *Planaria* and other forms. It seems to bear the stamp of a true regulative process for it brings the parts into the position which they must occupy in order to produce a bilaterally symmetrical whole.

During observations on *Planaria* in which the change is well-marked, the possibility suggested itself that it was primarily due, not to some internal factor operating in such manner as to produce the typical form of the species or an approximation to it, but rather to the locomotion of the animal in the direction of the longitudinal axis. It appeared probable that since the new parts were used for attachment and thus subjected to tension in the direction of the longitudinal axis they were gradually drawn out in this direction and so a symmetrical whole was produced. This view was supported by the fact that the change seemed to begin when the new part became functional. When the new tissue first appears in these forms it is apparently little used for attachment or at least without complete success. Within a few days, however, the specimens can be seen to adhere closely to the substratum by means of it, and it is at this time that the apparent change in the direction of growth first becomes conspicuous.

The question as to the effect of altering the direction of locomotion in pieces at once presented itself to me and fortunately I found in *Leptoplana* a favorable form for experiments of this kind. Short pieces from the body of *Leptoplana* containing the cephalic ganglia or a considerable portion of them move in circles

when one side of the body is cut away, since the axis usually becomes bent and there is nothing to counterbalance the effect of the cilia and muscular movements of the opposite side. The results of experiments with pieces of this kind demonstrated in a most satisfactory manner the correctness of my belief. Numerous experiments were performed, the results in all cases being unequivocal. In the following sections some of these experiments are described.

#### CIRCULAR LOCOMOTION AND REGENERATION IN PIECES OF HEADS.

A very satisfactory method of obtaining pieces which move in curves is that of separating the anterior end by a cut a short distance posterior to the cephalic ganglia and splitting this piece longitudinally in half at or near the median line. This method of preparation is illustrated by Figures 8 and 9. Figure 8 shows the

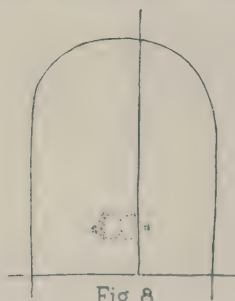


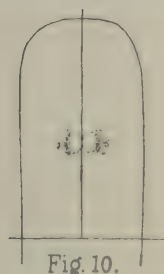
Fig. 8.



Fig. 9.

direction in which the cuts are made and Figure 9 the piece after contraction of the cut surfaces has taken place. It is evident from the latter figure that the contraction is an important factor in bringing about circular locomotion. The longitudinal axis of the piece becomes bent toward the cut side and movement in a straight line is impossible. The curve of locomotion approximates more or less closely the curve of the axis but does not necessarily coincide with it since the irregular form of the piece often alters the direction. The tissue giving rise to the new posterior region soon begins to show the effect of the direction of movement and the tail forms at an angle with the old parts. The description of the following series will serve to illustrate the course of regeneration.

I. August 31, 1903. A specimen of average size was prepared as shown in Figure 10. The greater part of the body was removed by a transverse cut about 2 mm. posterior to the cephalic ganglia and the anterior piece thus obtained was split longitudinally. In this case the longitudinal cut appeared to be coincident with the median plane, but the differences in behavior of the two pieces after section indicated that the left cephalic ganglion was injured to a greater extent than the right. After section the cut



surfaces soon contracted thus bending each piece into a curved form resembling Figure 9 and in both pieces locomotion diverged constantly toward the cut side, the pieces thus moving in circles, as indicated by the arrows accompanying the figures.

September 3: 3 days after section:

The pieces have assumed the forms shown in Figures 11 and 12. New tissue has appeared almost uniformly over the whole extent of the cut surface. The left piece (Figure 12) is considerably more contracted than the right piece (Fig. 11) and moves



somewhat more slowly. Numerous experiments to be discussed later have shown that with increasing injury to the cephalic ganglia the rapidity of movement and the degree of extension decrease, hence it is probable that the ganglion in the left piece has suffered greater injury than that in the right.

September 6: 6 days after section:

At this stage the regenerating posterior end has become distinct and the first traces of the new pharynx are visible (Figs. 13 and 14). The posterior outgrowth is directed toward the cut side and its axis coincides with the curve of locomotion. The new tissue is now functional to some extent, the tail being employed by both pieces for attachment.

September 20: 20 days after section:

The two pieces are shown in Figures 15 and 16. The axis of the new body is distinctly curved in both and the pharynx shows in each case some degree of curvature. In each the part of the ganglia removed is regenerating and in connection with it are a few eye spots. The small new ganglion was distinctly visible in the living specimens from the dorsal side. Both pieces continue to move in circles, though with a somewhat larger radius than before, the change being due to the development and use of the new tissue along the side of the head, which now evidently aids in locomotion and thus counterbalances in some degree the effect produced by the old parts. Portions of the margin of both the new tissue and the old can be extended antero-laterally and attached to the substratum, and tension is exerted upon the other parts by muscular contraction of these regions. But the power of the new portions is still much less than that of the old parts. Similarly, in consequence of the curvature of the old parts, itself due to the contraction following section, the effect of the cilia on these parts is such as to cause movement in a curved line which is not yet counteracted by the cilia on the new parts.

The larger right piece was accidentally injured at this time and its further history could not be observed.

October 12: 42 days after section:

Figure 17 represents the left piece at this stage. Considerable reduction in size has occurred, but the amount of regenerated tissue is relatively much greater than in Figure 16. The direction

of movement diverges less from a straight line than before, and correspondingly the angle between the longitudinal axis of the head and new body is decreasing. The new cephalic ganglion is nearly as large as the old, but the eyes are still less numerous in the new tissue than in the old. The regeneration of the lateral regions of the head has proceeded so far that anterior to the eyes the new portion is nearly as broad as the old.

The change in form of the regenerating lateral margin of the head is the most conspicuous feature of this stage (compare Figs. 16 and 17). It has now acquired almost its typical form. Moreover, the curvature of the longitudinal boundary between the new and old portions, *i. e.* the longitudinal cut surface, is decreasing. Observation of the movements of this piece at this stage showed that the functional activity of the regenerated margin of the head was very great. It was much used in locomotion, portions being extended anteriorly or antero-laterally, attached, and then contracted, thus drawing the body forward. Swimming movements were also often made, though short pieces of this kind do not succeed in swimming to any extent, being apparently unable to maintain their equilibrium in the absence of posterior parts of normal size. There can be little doubt that the functional activity of this region has brought about the change in form. Characteristic movements have produced a characteristic arrangement of the tissues. Moreover, the frequent extension of the margin anteriorly followed by attachment and contraction has undoubtedly aided in forcing the anterior part of the old tissue toward the left and thus straightening the outline of the cut surface.

October 24: 54 days after section:

As indicated in Figure 18 the changes described above continue. The piece moves still more nearly in a straight line than twelve days ago and the form is correspondingly altered. The present stage exhibits one interesting effect of the change in direction of the tension upon the tissues. During locomotion the margin at *x*, including both new and old tissue, is thrown into small wrinkles or folds, while the right side of the body is very evidently stretched. The folds are clearly the result of the altered direction of tension in the adjoining parts. The body grew out in a direction differing considerably from that in which

it extends at present and with the change in position the tension on the tissues at this point has decreased until now it has become pressure and these parts are "too long" for the position they must occupy under the altered conditions. A comparison of Figure 18, the form of the piece during locomotion, and Figure 19, the form during rest, when the parts are not subjected to longitudinal tension, renders it still more evident that the tension due to movement is the cause, not the effect of the change in form. When the piece is at rest the angle between the original axis and the axis of the new body is always greater than during locomotion and the folds at  $x$  disappear. In other words the change in direction of the new body does not precede but follows, and does not even keep pace with the change in direction of locomotion. These facts leave no room for doubt that the tension due to locomotion is the efficient factor.

In a previous section the fact was noted that locomotion in *Leptoplane* is chiefly ciliary when the animal moves quietly, but that when strongly stimulated the movements are to a large extent muscular. The same is true of these pieces. When stimulated only slightly they progress at a uniform rate, largely by means of the cilia, but under stronger stimulation the margins of the head are used in the manner described and the body is drawn forward by strong muscular contractions usually alternating on the two sides. A marked difference in direction between the two kinds of locomotion was observed in this piece and indeed in many other similar pieces. The direction of locomotion by muscular contraction was sometimes after strong stimulation in a curve to the left while that of ciliary locomotion was always toward the right. During locomotion the muscular activity of the right side of the head—the new tissue—appears to be greater than that of the old tissue on the left. In ordinary locomotion the muscular play of the margins of this part is much more conspicuous than on the left. Apparently the new parts are in a more active condition functionally than the old, and doubtless under strong stimulation are capable of more work. When the piece turns to the left after strong stimulation the difference in muscular activity between the two sides is much more marked, that of the right side being clearly much greater.



November 8: 69 days after section:

At this time the locomotion of the piece diverges only occasionally and then slightly from a straight line, except sometimes after strong stimulation when the piece turns to the left. The form is still more nearly symmetrical (Fig. 20). The folds which were visible at *x* in Figure 18 have disappeared in consequence of rearrangement or resorption of the superfluous parts (atrophy from disuse?), though when the piece turns to the left after strong stimulation folds appear temporarily in this region.

The longitudinal boundary between new and old tissue is now almost a straight line, *i. e.* the contraction of the cut surface which occurred after removal of the right side is now scarcely percept-

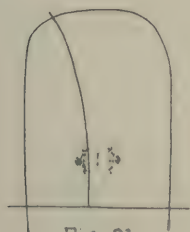


Fig. 21



Fig. 22.



Fig. 23



Fig. 24



Fig. 25.

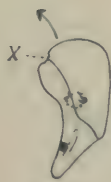


Fig. 26



Fig. 27.

ible. This change is probably, like the contraction itself, due primarily to an alteration in mechanical condition, *i. e.* the changes in mutual pressure and tension.

II. August 31, 1902. The body was removed by a transverse cut about 1 mm. posterior to the cephalic ganglia and the anterior piece thus obtained was split longitudinally (Fig. 21). The longitudinal cut passed a little to the left of the median plane and so through the left cephalic ganglion. In consequence of the contraction of the worm during the operation the course of the cut

was curved as shown in the figure. Only the piece on the right of the cut will be considered here. After section the cut surfaces contracted and the anterior end bent over so far that the outline of the anterior region became almost symmetrical (see the outline of the old tissue in Fig. 22).

September 3: 3 days after section:

Figure 22 shows the piece as it appears at this stage. The contraction has brought the two cut surfaces, originally at right angles into almost the same plane. New tissue has begun to appear but there is no marked difference in amount in different regions. That portion of the left cephalic ganglion which remained in the piece protrudes slightly from the cut surface and is indicated in the figure by deep shading. The piece moves in rather small circles as indicated by the arrow.

September 6: 6 days after section:

As indicated in Figure 23 the new tissue, probably the ectoderm, has united with the protruding portion of the left cephalic ganglion and is thus prevented from extending at this point. Anterior and posterior to this region growth has occurred and in a curious manner. It appears as if two posterior ends were forming, one from the lateral region of the head, the other from the posterior cut surface, both of them corresponding in direction to the tension resulting from locomotion. Both adhere to the surface to some extent, but the posterior one somewhat more firmly. Several factors combine to produce this peculiar condition: the piece has contracted in such a manner that the cut surface from which the left side of the head would normally regenerate faces somewhat posteriorly; the union between the protruding nervous tissue and the new tissue divides the growing region into two parts; and finally the tissue representing the left side of the head is just becoming functional so that its margin reacts to the contact of the substratum but does not yet extend anteriorly and contract strongly and aid in locomotion, being instead stretched postero-laterally since it adheres to the substratum until the forward movement loosens it. This condition shows very clearly how effective mechanical tension may be as a "formative factor."

September 10: 10 days after section:

Unfortunately the condition described above did not continue, for the ganglionic mass became separated from the right ganglion and remained united with the new tissue on its dorsal surface near the left margin (Fig. 24). In consequence of this change the regions of new tissue before separated are now continuous and the outline of the margin is rapidly undergoing alteration. Moreover, the new lateral tissue is now further developed functionally and its margin reaches forward, attaches itself and contracts in the characteristic manner, this assisting in the locomotion which consequently becomes slightly less curved in direction. Corresponding with this increase in characteristic functional activity is the convex outline of the lateral margin of this region. As long as it was being subjected to postero-lateral tension this portion of the margin was in part slightly concave like the sides of a growing tail or body (Fig. 23).

The mass of ganglionic substance affords a landmark which enables us to determine that the terminal portions of the body are formed first, a conclusion agreeing with that of various authors in regard to soft parts at least in other forms.

September 20: 20 days after section:

Figure 25 represents the condition at this stage. The direction of movement is still far from a straight line, though the curvature is decreasing. The curved body and pharynx require no special comment. The new lateral region of the head now functions very actively and a comparison of Figures 23, 24 and 25 shows that the curve of contraction of the original cut surface is becoming less marked, *i. e.* the old tissue is being pressed back toward the right at the anterior end by the active new tissue. Regeneration of the left cephalic ganglion is taking place.

October 12: 42 days after section:

The new lateral region of the head is now so active that it counterbalances the old part to a considerable extent and the direction of movement is less curved. Figure 26 shows the specimen at this stage. The change in form and the growth anteriorly of the lateral region is marked (compare Figures 25 and 26). Small folds at *x* during locomotion indicating the pressure exerted



by the functional activity of the new part. The angle between the body and the original longitudinal axis is decreasing, *i. e.*, the body is swinging into typical position.

October 24: 54 days after section:

At this stage the direction of locomotion approaches still more closely a straight line, and the form is correspondingly changed (Fig. 27). The small folds at *x* due to the pressure of the new lateral region against the old parts at the anterior end are still visible, and similar folds appear at *xx* in consequence of the change in position of the body. The left margin of the head shows great activity in the region where the lateral outgrowth is greatest, and frequently performs swimming or "flying" movements of some amplitude. The old portions, on the other hand, are less active. The regenerated cephalic ganglion is nearly as large as the other and eyes are present in connection with it.

Loss of the piece a few days later prevented completion of the record.



Fig. 28.



Fig. 29.



Fig. 30.



Fig. 31.



Fig. 32.

III. August 31, 1902. A specimen was prepared in the manner described for Series I and II, the longitudinal cut being made as nearly as possible in the median plane. Probably, however, it was actually a little to the right of the median plane, since, as in Series II, a part of what seemed to be the right cephalic ganglion protruded from the cut surface of the left piece, the part used.

Apparently the left cephalic ganglion was more or less injured by the operation, for during the first two weeks the piece showed

little motor activity. As regeneration proceeded, however, it began to revolve in circles scarcely greater in diameter than its own length, appearing almost as if revolving on a pivot.

The extreme curvature of the direction of locomotion renders the piece of interest and certain points require consideration. During the first period after section when locomotion was slight regeneration was almost uniform over the whole cut surface, *i. e.*, there was no marked extension of the portion representing the posterior region. Figure 28 represents the piece ten days after section. A comparison of this figure with Figure 24 the corresponding stage of Series II in which active locomotion has occurred during the ten days suggests the possibility that the tension or other conditions connected with locomotion may not only determine the direction of outgrowth of new tissue but may also affect the amount of regeneration, a point which will be discussed more fully elsewhere.

Within the next few days the piece began to move in the manner described above and twenty days after section appeared as represented in Figure 29. The axis of the regenerated portion forms an angle of more than ninety degrees with the original longitudinal axis. The protruding mass of nerve tissue has united with the new tissue and delayed growth at that point. The rapid change of form which has occurred in the new tissue during ten days indicates the marked effect which use of the parts exerts upon regeneration.

Twenty-two days later, forty-two days after section, the form was much the same. Figure 30 represents the piece during ordinary locomotion. Frequently the piece assumed the form shown in Figure 31 in which the tip of the tail was overlapped by the lateral margin of the head. Figure 32 represents the form assumed when the piece attached itself by the tail and contracted, drawing itself backward; in this condition folds appear at  $x$ , indicating that pressure instead of tension occurs in that region.

About ten days later the piece died without further changes. This case differs from the preceding in that no marked change in the direction of the body-axis occurred during the whole history, although the piece lived as long as many others in which the

change occurred. The continued circular locomotion is of course directly responsible for the absence of change in form and this in turn may be due to the delayed regeneration of the right cephalic ganglion and consequent imperfect coordination of the new lateral margin. As a matter of fact the right lateral margin of the head appeared much less functionally active than in the other cases described. Regeneration of the ganglion was delayed by the presence of the old ganglionic tissue which did not lose its connection with the left ganglion until about four weeks after section. And finally this long-continued attachment of the injured ganglionic tissue to the left ganglion is doubtless to be ascribed to the fact that scarcely any locomotion occurred during the first two weeks after section, so that the new tissues with which the ganglionic tissue was united were not subjected to tension which would aid in removing this tissue from the region where its presence interfered with regeneration. I have no doubt that had the piece lived sufficiently long before exhaustion occurred, the right lateral margin of the head would have acquired its characteristic activity and so would have counterbalanced the motor effect of the old tissue, thus bringing about the change in direction of the body-axis which occurred in Series I and II.

In all of the cases described thus far the transverse cut surface, originally posterior, becomes oblique in consequence of the contraction and in most cases the outgrowth of new tissue forming the body occurs in a direction nearly perpendicular to this surface, though there is considerable variation in different cases. Thus in Figures 15 and 16 of Series I the angle between the axis of the new body and the cut surface is somewhat more than  $90^\circ$ , while in Figures 23, 24 and 25 of Series II it is approximately  $90^\circ$ , and in Figures 28, 29 and 30 of Series III it is again more than  $90^\circ$ . These cases therefore are open to the objection that the direction of growth may have been determined in some degree by the direction of the cut surface rather than by the tension due to movement, for it is a well known fact that in many cases regeneration takes place chiefly at right angles to the cut surface. Although I did not consider this objection valid I prepared other series in which the posterior cut surfaces of the pieces were



strongly oblique (Fig. 33) in order to obtain experimental evidence on the question. A few cases from one of these series are described. These cases show that the direction of regeneration



Fig. 33.



Fig. 34.



Fig. 35.



Fig. 36.

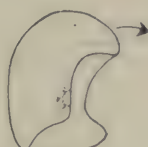


Fig. 37.



Fig. 38.



Fig. 39.



Fig. 40.



Fig. 41.



Fig. 42.

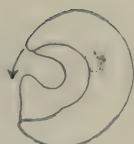


Fig. 43.



Fig. 44.



Fig. 45.

is not determined, except of course in the early stages, by the surface or surfaces from which it occurs.

IV. September 3, 1902. Four large specimens were cut in the manner represented in Figure 33. By this method the relation between the plane of the cut surface and the direction of locomotion is different in the right and left pieces.

Two pieces of each set are described, the others showing no additional features of importance.

1. A piece from the left side (see Fig. 33): Figure 34, seven days after section; Figure 35, twenty days after section; Figure 36, thirty days after section. In this case the cut surfaces remained nearly in their original relations and the piece was not greatly bent. Consequently the curvature of the axis and of the direction of locomotion was not as great in many cases. The angle at which the body appears corresponds with the direction of locomotion, but the axis of this region is far from perpendicular to the posterior cut surface. In later stages this specimen became symmetrical.

2. A piece from the left side: Figure 37, seven days after section; Figure 38, twenty days after section; Figure 39, thirty days after section. This piece became so bent during contraction that the posterior cut surface faced somewhat toward the right instead of to the left as originally (compare Figs. 33 and 37) and the direction of locomotion was correspondingly curved. The longitudinal cut was a little to the left of the median plane, thus injuring the left cephalic ganglion to some extent. The piece was consequently less active in locomotion and the right margin did not acquire full functional activity as soon as in many other cases. There was therefore no marked change in the direction of locomotion and the curvature of the axis persisted to a great extent up to the time of death. In this case also it is evident that the outgrowth forming the posterior region is not perpendicular to the posterior cut surface.

3. A piece from the right side of the head (see Figure 33): Figure 40, seven days after section; Figure 41, twenty days after section; Figure 42, thirty days after section. In this case the contraction of the piece, though not great, brought the two cut surfaces almost into line. The curvature of the regenerating body is clearly shown in Figure 41. The outgrowth is more

nearly perpendicular to the posterior cut surface in this case than in the two preceding cases, but this is to be expected from the position of the latter. In this piece the regeneration of the left cephalic ganglion was not delayed, the left margin of the head became functionally active within a month after section (Fig. 42) and reduction of the curvature began and was completed before death.

4. A piece from the right side of the head: Figure 43, seven days after section; Figure 44, twenty days after section; Figure 45, thirty days after section. The longitudinal cut in this case injured the right ganglion to some extent. The piece became greatly bent and simply revolved within a space little greater than its own size. The posterior cut surface was brought into line with the longitudinal surface. The posterior region grew out toward the anterior tip of the head, but not at right angles to the plane of the cut surface. Within the month the left cephalic ganglion was partially regenerated and the left margin of the head attained some degree of functional activity thus reducing the curvature of locomotion and the axis of the body began to straighten (Fig. 45). The piece did not, however, attain anything like symmetrical form before death.

A comparison of these four cases renders it sufficiently evident that the angle between the plane of the posterior cut surface and the regenerating body may vary greatly, while, on the other hand, the relation between the direction of the outgrowth and the direction of locomotion is evident.

In the cases described the relation between the functional activity of the regenerating margin of the head and the direction of locomotion has been pointed out. Attention has also been directed to an apparent relation between the development of this functional activity and the regeneration of the cephalic ganglion of that side. The relation of the nervous system to regeneration will be discussed elsewhere, but the fact may be noted here that the presence or absence of the one ganglion appears to affect the regeneration of the lateral margin of the head, but not that of the posterior region. The latter may be formed in the usual manner when only one ganglion is present, though it is usually not as



long under these conditions as when both are present. I think there is little doubt that the difference is connected with the functional activity of the parts. Each region develops its characteristic form only as it is used in the characteristic manner. In all cases marked growth anteriorly and laterally of the new margin of the head has been observed as soon as the animal begins to use it in the manner characteristic of these regions, while the case represented in Figure 23 shows that so long as this region is not used in the ordinary manner it may develop posteriorly. The posterior region of the body can perform its functions to some extent in the absence of the cephalic ganglia as will be shown elsewhere, and moreover, in the cases under consideration the regenerating posterior region is undoubtedly innervated from the part of the nervous system present. It therefore performs its usual functions, though perhaps less perfectly, before the other ganglion regenerates, and its development proceeds in the typical manner. To put it briefly, the margin of the head develops a characteristic form because used in a characteristic manner, and the body develops a different form because it is used differently. The preceding experiments are sufficient to show that mechanical tension is an important factor in morphogenesis in these animals. No one would admit more readily than myself, however, that many other factors may be concerned here and that in other cases the factors may be wholly different.

Attention has been called in several cases to the apparently greater functional activity of the regenerated parts as compared with the old in later stages. This difference indicates, I believe, a real physiological difference. The old portion decreases in size in consequence of loss of material while the new parts increase in absolute size in the earlier stages and in relative size in later stages. It is not at all improbable that the functional condition of the reduced old part differs widely from that of the new part. The former, reduced to a fraction of its former size, is certainly less plastic mechanically and probably less sensitive to stimuli. The new part is to be regarded as possessing the qualities of a young and growing organism, the old on the other hand as approaching exhaustion. The difference observed in functional ac-

tivity between new and old portions agrees well with the fact already mentioned in the section on "Regulation, Nutrition and Use of Parts," that in starving pieces the old part usually dies and disintegrates before the new, which may live and remain apparently healthy for several days after the loss of the old part.

#### CIRCULAR LOCOMOTION AND REGENERATION AFTER OBLIQUE SECTION.

Another method employed for bringing about circular locomotion was that of making oblique cuts at various levels posterior to the cephalic ganglia. The results obtained by this method are in some respects less striking than those described in the preceding section, but since the cephalic ganglia are not injured in any way by this method, a possible objection to the preceding series is rendered invalid.

The change in direction of the longitudinal axis of the regenerating tissue arising from a posterior oblique cut surface has been mentioned (see also Figures 5-7). In this case the direction of locomotion was not altered by the cut, and, as might be expected, the axis of the new tissue soon became coincident with that of the old. In the course of similar experiments I found, however, that if the cut were very oblique the contraction of the cut surface following the operation might bring about circular locomotion. Figure 46 represents the manner in which such a cut is made, and Figure 47 the piece after contraction. It is evident that contraction produces a marked curvature in the longitudinal axis, and therefore the piece in advancing turns constantly toward the cut side. It was found necessary to make the cut in the anterior region of the body in order that markedly circular locomotion might occur, because if the cut were made near the middle of the body or in the posterior half, the part of the body in which the axis was not affected by the cut was so long that the bilaterally symmetrical impulse to movement from this region nearly or quite counterbalanced the effect of the bent portion, and the regenerating tissue grew out in the direction of the old axis. In all cases where the circular locomotion was well marked the cut was made either just anterior to the pharynx as



Fig. 46.



Fig. 47.



Fig. 48.



Fig. 49.



Fig. 50.



Fig. 51.



Fig. 52.

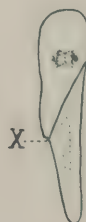


Fig. 53.



in Figure 46 or through its anterior portion. Within certain limits the circular locomotion is more marked as the obliquity of the cut increases, the reason being clear. If, however, the cut be nearly longitudinal the slender strip on the longer side is likely to roll up and may act as a drag, thus complicating locomotion and delaying or preventing typical regeneration.

Figures 48-53 illustrate the history of a piece cut in the manner indicated in Figure 46 and moving in a curve toward the right. Even as early as three days after section (Fig. 48) the new tissue is symmetrical with respect to the cut surface, evidently in consequence of the effect of locomotion. Figure 49 shows the condition six days after section. Here the curvature of the new tissue is becoming evident. In Figure 50—twelve days after section—the curvature of the regenerating part is still more conspicuous. Twenty-eight days after section the tissue has acquired the form shown in Figure 51. Now that the regenerated tissue has attained a considerable length and its posterior region constitutes the chief organ of attachment some degree of straightening occurs. The manner in which this takes place is shown in Figures 52 and 53, both of which represent the same stage—fifty-eight days after section. Figure 52 shows the piece in ordinary locomotion. Here the margins as well as the tip of the tail, or frequently only the margins or certain regions of them, adhere to some extent, and the resulting tensions cannot cause straightening of the longitudinal axis since they follow approximately the same curve. Frequently, however, only the posterior end of the body adheres, or the margins of the head and the posterior end, and at such times the piece assumes temporarily the form of Figure 53, in which the axis is nearly straight. The longitudinal tension to which the body is subjected straightens it, but at the same time bends the contracted posterior part of the old tissue to the left so that the outline becomes convex at the left of this region. At the same time small folds appear at  $x$ , indicating that in this region the tissues are subjected to pressure instead of tension. This position is never maintained for any length of time, and as soon as the tension ceases the piece resumes the form of Figure 52. There can be no doubt, however, that if this position is taken sufficiently

often straightening will occur. This I believe is the chief factor in the change from the curved to the straight bilaterally symmetrical form. This piece died after sixty-six days without having become completely symmetrical.

The history of other pieces prepared in a similar manner is essentially the same, though, as has been mentioned, the degree of curvature of the new tissue varies within certain limits with the obliquity of the cut and with the level at which the cut is made. The reason for variation in curvature with the angle of the cut lies in the fact that the more oblique the cut the more the axis of the piece is bent and consequently the greater is the curvature of locomotion.

As regards the level, the curvature of the new tissue decreases as the distance of the cut from the anterior end increases, because the region of the body in which the axis is not bent by the contraction of the cut surfaces increases and counteracts the asymmetrical motor effect of the bent region more and more completely. This variation of curvature with the level of the cut renders it evident that the cut surface itself has little influence upon the direction of growth except in the earliest stages, for a piece cut at a given angle near the anterior end, *e. g.* as in Figures 46-53, will give rise to new tissue with a marked curvature, while in another specimen cut at the same angle but farther posteriorly the new tissue will show much less curvature while in still another, cut at the same angle in the posterior pharyngeal region, the new tissue will grow out in the direction of the longitudinal axis or will very soon acquire this direction, simply because the direction of locomotion is curved only very slightly or not at all. In each case the direction of outgrowth coincides with the line of locomotion.

#### GENERAL CONSIDERATIONS.

The bearing and the significance of the experiments described is sufficiently clear to render extended discussion unnecessary. They may, I think, be regarded as demonstrating the fact that the extended form of the body in *Leptoplana* is determined in large degree by mechanical conditions. It is difficult to describe ac-

curately the continually changing movements of these animals, but I think no one who actually observes the pieces can fail to be convinced of their importance in determining form. It is true of *Leptoplana* as of *Stenostoma* (Child, '02) that it has no "normal form," *i. e.*, no definite hard and fast form inherited and developing in the individual independent of physical conditions. What these animals do possess is a capacity for certain kinds of activity. These are given potentially in the chemical and physical structure of the protoplasm, which to my mind represents rather capacity for functional activity in the broadest sense than form. As my experiments prove, certain elements of form in the morphological sense develop incidentally as the result of functional activity in a given environment. These elements have been commonly regarded as typical and determined by heredity because they are common within certain limits of variation to all individuals of a species, but when we consider that under natural conditions both functional activity and environment are essentially similar in different individuals of the species the reason for likeness in these form-elements becomes clear. It is only when we can alter the functional activity as I have done experimentally in the case of *Leptoplana*, or the environment, as I succeeded in doing for *Stenostoma* (Child, '03a) that the dependence of these elements of form upon these two factors becomes clearly evident.

But these experiments concern only morphological characters of a certain kind. Experiments of others have already shown that "formative factors" are many and various, and generalizations from the consideration of a single group of characters are unsafe. It will never be possible to explain form on the basis of a single principle. All the complex activities of which organisms are capable are "formative factors": when we can view all of these in their complex interrelations and know the part which each plays, then and only then shall we "understand" organic form.

The relation between form and heredity has never been satisfactorily determined. With the advance in our knowledge the fact becomes more and more evident that the organism is not merely a complex of structural elements ready made by heredity for certain functional activities, but rather a complex of ac-



tivities in consequence of which morphological structure develops. Physical and chemical structure of protoplasm must not be confused with morphological structure: the distinction between the two is important though often overlooked. As regards the individual the former represents capacities for activity, *i. e.*, for transformation and transference of energy, or in short, functional activity in the broadest sense. Form in the morphological sense, is the combined result of this activity and the environment, external or internal. According to this view, it is functional capacity that is inherited rather than form: heredity is, strictly speaking, a physiological and not a morphological problem.

#### SUMMARY.

1. Locomotion in *Leptoplana* is accomplished by two methods, swimming and creeping. In swimming the lateral regions of the anterior part of the body perform undulating movements in a dorso-ventral direction. Creeping movements are both ciliary and muscular, the muscular movements consisting chiefly of an alternate extension anteriorly or antero-laterally of the margins of the head, adhesion to the substratum and muscular contraction, thus drawing the body forward. In creeping the margins and posterior end of the body are used as organs of attachment.
2. In consequence of the typical movements the tissues of the body are subjected to typical mechanical tensions and pressures which constitute formative factors.
3. The effect of these mechanical conditions upon the tissues is at least in part directly mechanical, but they may also act as physiological stimuli to growth (formative stimuli).
4. The effect of the mechanical conditions incident to locomotion may be demonstrated experimentally by various methods. The method described in this paper consists in making the pieces of such a form that the direction of locomotion becomes curved instead of straight. In these experiments the regenerating part grows in the direction of the principal tension, even though this form an angle of  $90^\circ$  with the typical direction of growth.
5. The experiments lead to the conclusion that in *Leptoplana* the regions of the body develop in a characteristic form because they function or attempt to function in a characteristic manner.

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## SELF-FERTILIZATION INDUCED BY ARTIFICIAL MEANS.

BY  
T. H. MORGAN.

It has long been known that the pollen of some plants will not fertilize the ovules of the same plant. The cause of this impotence has not yet been detected.

It is also known that pollen from another plant is often prepotent in those cases where normal self-fertilization may occur. It has further been shown, especially by Darwin, that the offspring from self-fertilized ovules are in general not so vigorous as those from cross-fertilized ones.

There are here two problems, which, even if they should prove to be fundamentally related, can be most profitably examined separately;—first, the problem of the inability of the male element to fertilize the female germ-cells of the same individual; and, second, the effect of self-fertilization (in those cases in which it occurs) on the offspring. Both problems appear to be within the range of experimental examination.

There are only a very few cases known amongst animals where conditions similar to those in plants have been found to prevail, although very few hermaphroditic animals appear to have been examined in this respect. Close inbreeding, which is commonly supposed to bring about deterioration in some cases, is perhaps not very dissimilar to self-fertilization. Whether in the case of inbreeding there is ultimately a loss of power to fertilize the egg, or whether the egg fails to develop after it has been fertilized, has not, so far as I know, been determined.

Castle discovered in the ascidian, *Ciona intestinalis*, a case apparently similar to those in plants. The eggs are generally incapable of self-fertilization, yet can be readily cross-fertilized; *i.e.*,

the spermatozoa of an individual will not fertilize the eggs of that individual, but have the power to fertilize the eggs of any other individual.

My object in undertaking a study of this problem was, in the first place, to determine if possible the nature of the conditions that prevent or interfere with self-fertilization; and in the second place, I was not without hope of being able to find some way in which self-fertilization could be artificially induced. As will appear in the sequel, these two questions are not two sides of the same problem; for, while it has been possible to discover the means of bringing about self-fertilization, it still remains to be definitely determined what conditions in the egg normally prevent the entrance of the spermatozoa of the same individual.

Since Castle's observations had shown that the ascidians offer favorable material for a study of this sort, I first turned my attention to this group, using the three most available species found at Woods Hole, or in the vicinity; namely, *Ciona intestinalis*, *Molgula manhattensis*, and *Cynthia partita* (*Styela* sp.). The work was done while holding the Bryn Mawr Table at the Marine Biological Laboratory, from June to September, 1903. Owing to the scarcity of *Ciona* I have not been able to work out completely a number of important problems connected with one of the two main questions that I examined. In the near future I shall hope to complete this side of the investigation.

#### EXPERIMENTS WITH *CIONA* INTESTINALIS.

The ovary of *Ciona* is a sac-shaped body of fair size lying loosely attached in the coil of the intestine. It can easily be removed without cutting into the testis. Its lumen contains some of the ripe eggs, but the majority of these are in the oviduct. The oviduct can readily be opened and the eggs set free without cutting into the vas deferens, which follows a course parallel to the oviduct. If the animal is kept isolated for 24 hours the oviduct becomes greatly distended with eggs, and after another 24 hours even more eggs may have accumulated. The eggs are laid normally in the early morning, at dawn, and Castle has recorded that *Ciona* deposits its eggs and sperm with the regularity

of the rising sun. The rough handling incidental to removal and isolation appears to cause *Ciona* to retain its eggs for several days. The individuals to be used were isolated, as a rule, from 24 to 48 hours, and in most cases were rinsed in fresh water before opening. It was not found necessary to boil the water; for check experiments showed that eggs left to themselves were never fertilized by stray spermatozoa in the sea-water. Since *Ciona* deposits its eggs only in the very early morning, the chances are very slight that functionally active spermatozoa would be present in the sea-water in the late morning and in the afternoon when the experiments were carried out.

The eggs of *Ciona* are surrounded by a rather thick membrane. Standing out like broad spikes over the surface of the membrane, and forming a beautiful aureole around the egg, are the transparent follicle cells, each with a shining drop in its outer end.

A number of preliminary experiments confirmed Castle's conclusion that self-fertilization is rarely possible in *Ciona intestinalis*. The evidence, however, on which Castle based this conclusion is not altogether satisfactory, since he records many cases in which self-fertilization occurred. Instances are cited in which isolated individuals gave 90, 25, 16, 5, 4, 0 per cent. of self-fertilized eggs. Castle supposes that, in the first of these cases at least, the spermatozoa of one day fertilized the eggs of the next, but it has not been shown that the spermatozoa have this power if left so long in sea-water. The same individuals that had been used for these isolation experiments were killed (after being washed in 90 per cent. alcohol), and the eggs and sperm of each taken out and mixed together. The results gave 50, 4, 1,  $\frac{1}{3}$ , 0 per cent. of self-fertilized eggs. The same experiment repeated with fresh individuals gave 50,  $12\frac{1}{2}$ , 10, 5, 2, 0 per cent. of self-fertilized eggs. From these figures it is clear that in some cases a considerable amount of self-fertilization occurred, unless there was some source of error in the experiment. In fact, Castle believes that in those cases where a large number of eggs were fertilized there was some contamination. My own results with *Ciona* have never given so large a percentage of self-fertilized eggs, and I am inclined to attribute this result in part to the



precaution that I took to isolate the individuals the day before they were to be used. I have rarely seen more than from 1 to 10 per cent. of self-fertilized eggs segment, and in the greater number of cases not a single egg segmented. On the other hand I found, as did Castle, that as a rule 100 per cent. of cross-fertilized eggs develop, to which statement I should add, provided the spermatozoa are in "good" condition.

What is the meaning of these remarkable facts? Why do not the sperm fertilize the eggs produced by the same individual, and yet fertilize those of any other individual? A number of possibilities readily suggest themselves, and since the following pages record an attempt to test these suggestions they may be briefly mentioned here:

1. That the spermatozoa are not made sufficiently active by secretions from the eggs of the same individual, but by those from the eggs of any other individual.

2. That the spermatozoa are not "attracted" to the eggs of the same individual.

3. That the egg contains, or secretes some substance that lessens the activity of the spermatozoa of the same individual.

4. That some mechanical difficulty prevents the spermatozoön from entering the egg of the same individual.

5. That even if the spermatozoön enters, it can not fertilize the egg of the same individual, in the sense of causing the egg to begin to develop.

In order to discover if the lack of power to self-fertilize the eggs is due to the absence of some substance around the eggs that excites the spermatozoa, the following experiment was carried out. The eggs of an individual (A) were taken from the oviduct. Similarly the eggs of another individual (B) were also taken out. Then the ovary of (A) and that of (B) were crushed separately, and a little sea-water was added. The eggs of (A) were then allowed to soak in the crushed ovary extract of (B) and those of (B) in the extract of (A). After a short time the sperm of (A) with a little water was added to the (A)-eggs, and the sperm of (B) to the (B)-eggs. If the sojourn of the eggs in the extract of the ovary of another individual has

the postulated effect, or if the presence of the extract of the ovary of another individual has the postulated effect on the sperm, fertilization ought to have occurred. The results showed, however, that fertilization did not take place.

This experiment was performed four times, giving eight sets in all. In six of these sets not a single egg segmented. In two others a very few eggs segmented (6 per cent. in one, 5 per cent. in the other), but this sometimes occurs in self-fertilized eggs not treated in any special way. Moreover there may have been contamination in the latter case.

Another experiment similar in some respects to the last was also carried out. The heart of one individual was opened and the blood collected. The eggs of another individual were put into this blood and allowed to stand. Later, sperm of the same individual was added in sea-water, but no fertilization occurred in one set and only one per cent. in the other. Check eggs were also kept in this experiment to make certain that no sperm had accidentally gotten into the blood. That none were present was shown by the fact that no fertilization took place. It is evident from this experiment that self-fertilization can not be brought about by soaking the eggs in the extract from the ovary or in the blood of another individual, although the somewhat high percentage of self-fertilized eggs that segmented in two cases after treatment with the ovarian extract may have resulted from the influence of the extract on the spermatozoa.

If the spermatozoa are excited to greater activity by the presence of the eggs of another individual it seemed not improbable that this might be directly observed. Therefore, I placed some of the sperm with the eggs of another individual and more of the same sperm with the eggs of the same individual, and compared the two preparations under the microscope. The spermatozoa of *Ciona* are not very active as a rule, nor do they accumulate in crowds around the eggs, as they do in many other animals, or at least not to any marked extent. It seemed to me in both cases that sometimes the spermatozoa were more active immediately in the vicinity of the eggs, and in the spaces between the follicle cells, but as they also show the same activity around

pieces of the tissue of the body of the same or of another individual I have not laid much stress on this observation, or accredited the results to the presence of an exciting substance. At times I have thought that the spermatozoa were more active around the eggs of another individual than around the eggs of the same individual, but as there is no very accurate means of determining their relative motility, unless very marked, I should not wish, as yet, to give a final answer to this question. It is certain that there is no such great difference in the behaviour of the spermatozoa in the presence of the eggs of the same and of another individual as to suggest that the difference in the result is connected with this factor. And even if this were the case, the influence probably extends for only a short distance from the surface of the egg, as the following experiment shows.

The eggs were taken from the oviduct, great care being taken not to injure the sperm-duct. The eggs from another individual were collected in the same way. An equal number of eggs from each were put together and fertilized with the sperm from one of the individuals. In another dish another lot of the same eggs were mixed half and half, and these fertilized with the sperm from the other individual. In each of these two sets half at least of the eggs should be fertilized by the other sperm, but half should not be fertilized unless the eggs of one individual exert some influence that causes the sperm to fertilize the eggs of the same individual also. It was found that only about half of the eggs were fertilized. This result shows that the fertilization is probably not due to some substance set free by the eggs that acts on the sperm or at least that if such a substance is set free its action is confined to the immediate vicinity of the egg. The experiment does not show, however, whether the egg, or its membranes, may not contain some substance that prevents the spermatozoa from entering the eggs of the same individual. Even if such a substance is set free from the eggs it may not have had time in my experiment to accumulate sufficiently in the surrounding water to have prevented the spermatozoa from fertilizing the other eggs, which may be quickly entered. This view can be tested by letting eggs stand in a small amount of water for :



long time, then taking out some sperm from the same individual, first making it active by placing it in sea-water, and then putting it into the water in which the eggs have stood. On the hypothesis these sperm should soon be brought to rest, and if then the eggs of another individual are added, they should not be fertilized, or at least not in the same proportion as when the sperm is taken directly from the oviducts, put into sea-water, and then added to the eggs.

THE INFLUENCE OF ETHER ON CROSS-FERTILIZATION.  
EXPERIMENTS WITH *CIONA*.

My first experiments with ether were made in order to determine whether when eggs are etherized it might not be possible to self-fertilize them. The results turned out somewhat differently from what I had anticipated, for although I found that it was possible to self-fertilize the eggs in ether-solutions, the result seemed to be due to the action of the ether on the sperm rather than on the eggs.

The experiment was first made with *Cynthia*, which in most cases has very sluggish spermatozoa. I observed that the first effect of the ether was to make the sluggish sperm very active, and even greatly quickened the activity of already active sperm. Furthermore I found that spermatozoa that scarcely moved at all in sea-water became active in the ether-solutions. Finally I found that in ether-solutions of certain strengths the eggs of *Cynthia* and of *Ciona* could be self-fertilized. The eggs behave in this respect so capriciously that I was obliged to carry out a large number of experiments in order to determine the conditions that lead to the self-fertilization of eggs in ether-solutions. The outcome was only partially satisfactory, but the experiments opened up a field for research, in which it may be possible to obtain further results of interest.

The experiments with ether were carried out as follows: At first I used a nearly saturated solution of ether and diluted it a half, or a fourth, etc. In the later experiments I used solutions of known strength. It was found by trial that the solutions were effective between 0.25 and 5 per cent. Some of the results may now be given in detail.

*Experiment I.* The eggs were removed from an individual that had been isolated 20 hours. The sperm was also taken out, and, together with the eggs, was put into ether-solutions, 5, 2, 1, 0.7, 0.5 per cent. in sea-water. After 5 minutes, and again after 10 minutes, the eggs were removed to pure sea-water. The eggs were injured by the ether in the strongest solution, but nevertheless one segmented. In all of the other solutions about 80 per cent. of the eggs divided; the most in the weaker solutions.

*Experiment II.* In this experiment the eggs and the sperm were put together into ether solutions of 5, 2, 1, 0.7, 0.5 per cent. Some of the eggs were transferred to water after 5 and 10 minutes, but others were left in the solutions. In the strongest solution the eggs were killed. In the others the following results were obtained:

Ether	Eggs segmented 5 minutes in ether.	Eggs segmented. 10 minutes in ether.
	20 per cent.	25 per cent.
2. per cent.	20 per cent.	25 per cent.
1. "	5 "	75 "
0.7 "	2 "	2 "
0.5 "	10 "	5 "

It is clear that the stronger solutions gave the best results, and that ten minutes immersion was better than five minutes. None of the eggs that were left in the ether-solutions segmented. This does not mean that they were not fertilized, but that the ether so injured the eggs after a long immersion, that they failed to develop. Several check experiments were also made in this case. In one the eggs were not self-fertilized but were put into a 5 per cent. ether-solution, and transferred after ten minutes to sea-water. They did not segment, nor did a few that were left behind in the ether solution. In another check series the eggs were not fertilized, and were left in sea-water. None segmented, which shows clearly that the ether in the preceding experiment was in some way responsible for the self-fertilization of the eggs. It should also be recorded that tadpoles developed from all the fertilized eggs that had been in the ether-solutions.

*Experiment III.* The eggs and sperm of the same individual

were put into ether-solutions of 3, 2, 1, 0.7, 0.5 per cent., and were removed to sea-water after 10, 20 and 30 minutes.

Ether	10 min.	20 min.	30 min.
3. per cent.	20 per cent.	20 per cent.	0 per cent.
2. "	33 "	17 "	90 "
1. "	50 "	25 "	10 "
0.7 "	50 "	20 "	0 "
0.5 "	20 "	20 "	12 "

The table shows that eggs segmented in all of the solutions, best however in the stronger solutions, although in one case the eggs became so injured by the ether that they did not develop further than the segmentation stages. In a check series, in which the self-fertilized eggs were put into sea-water, about ten per cent. of the eggs segmented. There may have been some source of contamination, or else, and this seems more likely since the individual had been isolated 20 hours, self-fertilization took place on a larger scale than usual.

*Experiment IV.* Eggs and sperm were mixed in ether-solutions of 4, 2, 1, 0.5 per cent.

Ether	2 min.	4 min.
4.	0	0
2.	40	1
1.	35	30
0.5	5	1

These results show that the 4 per cent. solution was too strong, while the 0.5 per cent. solution appears to have been too weak. The injurious action of the 4 per cent. solution appears to have been mainly on the sperm rather than on the eggs, for these eggs after they had been in sea-water 4 hours were capable of being cross-fertilized, and 25 per cent. of them developed.

*Experiment V.* Eggs and sperm were put into a 2 and into a 0.5 per cent. ether-solution and removed after 5 minutes.

Ether	5 min.
2.	90
0.5	90



It is interesting to note in this case, in which so large a percentage of the eggs were self-fertilized in ether, that of several hundred eggs of the same individual, to which sperm was added, but which were kept in sea-water, not one segmented. It was also found, and will be referred to again later, that when the sperm alone was put into a 2 per cent. solution of ether for five minutes, and was then added to eggs of the same individual in sea-water, 70 per cent. of the eggs segmented.

*Experiment VI.* In this experiment the eggs and the sperm were put into ether-solutions of 2 and of 0.5 per cent. and removed after ten minutes. In one lot the eggs were self-fertilized, in the other they were cross-fertilized.

Ether	Self-fert.	Cross-fert.
	10 min.	10 min.
2.	0	100
0.5	0	100

In this case although no self-fertilization took place, all the crossed eggs which had also been in the ether-solution developed, showing that the solutions have no baneful effect on cross-fertilization. The lack of self-fertilization shows that the sperm were not sufficiently acted upon by the ether-solutions employed to effect self-fertilization.

*Experiment VII.* This experiment shows how slight a difference in the conditions may cause great differences in the result. The individuals had been isolated 48 hours. One lot of self-fertilized eggs was kept in water and allowed to stand there 20 minutes. The eggs with the surrounding sperm were then put into ether-solutions of 2, 1 and 0.5 per cent. for 20 minutes, and then returned to sea-water. None of these segmented. Another lot of eggs from this individual were mixed with sperm of the same individual and put into a 2 per cent. ether-solution for ten minutes and then carried back to sea-water. Here 95 per cent. of the eggs segmented. On the other hand some of these same eggs taken from the ether after 5 minutes did not divide. The following experiments were also carried out with other self-fertilized eggs of the same individual.

Ether	15 min.	30 min.
2.	45	0 (only 3 eggs)
1.	30	40

*Experiment VIII.* The eggs and the sperm of one individual were mixed in ether-solutions of 4, 3, 2, 1, 0.7, 0.5 per cent., and removed after 10, 20, 30, 60 minutes to sea-water. It was noticed that the spermatozoa were very sluggish in sea-water, and although somewhat more active in the ether solutions, yet their activity was not marked. Of the eggs, which appeared to be in excellent condition, only three segmented, two in the 1 per cent. (20 minutes) and one in the 0.5 per cent. (20 minutes.)

The eggs that had not segmented after the ether treatment were fertilized, after they had stood 6 hours, with sperm from another individual, and three quarters of an hour later nearly all had divided normally into two cells. It was observed that the spermatozoa of this second individual, used for cross-fertilization, were also very inactive in their own fluid. They seemed to be more active in the extract from the ovary of the first individual. This ovary had also stood 6 hours in sea-water.

At the same time another experiment was made in which the eggs of another individual, that had been isolated for 24 hours, were put into ether solutions of 3, 2, 1 per cent. for 10, 20, and 60 minutes, and then returned to sea-water. None of these eggs divided, except one in the 3 per cent. solution (60 minutes). It was observed in this case that the sperm was inactive even in the ether-solutions, but nevertheless this same sperm, not in ether, cross-fertilized the eggs of the other individual in the preceding series, and also, as stated, appeared to be somewhat active in the extract of the ovary of the other individual.

*Experiment IX.* The eggs and sperm together were put into ether-solutions of 5, 4, 0.7 per cent. The sperm were active even in sea-water. The individual had been isolated for two days.

Ether.	10 min.	20 min.	30 min.
5.	0	12	0
4.	10	5	(7 out of 8 eggs)
0.7	12	10	(9 out of 10 eggs)

There was another series in this set in which the ether was stronger (about one-half saturated). None of the eggs from this solution segmented, but they became filled with clear spots. In another check series, self-fertilized but kept in sea-water, none of the eggs developed.

*Experiment X.* Sperm alone was put into ether solutions of 6, 4, 1, 0.5 per cent. It was removed (along with some of the surrounding fluid) and added to the eggs after 2 and 10 minutes.

Ether	2 min.	10 min.
6.	0	0
4.	0	0
1.	20	10
0.5	4	50

It appears from this experiment that it suffices to put only the sperm into the ether-solutions to bring about self-fertilization, but it should not be overlooked that a certain amount of the ether is carried over with the sperm when the latter is added to the eggs. The amount, it is true, will be small, since the eggs stand in water which further dilutes the ether, but so long as this source of error is present, and it is very difficult to remove it entirely, the result does not show conclusively that the ether acts on the sperm alone, although I think this is the more probable interpretation.

A check series of experiments was also made in which both eggs and sperm were put into solutions of the same strength as those given above, for 15 minutes and then removed to water.

Ether.	15 min.
6.	0
4.	0
1.	100 (but only ten eggs present.)
0.5	90

It is evident from both of the foregoing tables that only the weak solutions were effective, and from the first table it appears that this must have been the result of injury to the sperm. It can easily be seen that the eggs also are killed in a few minutes by a 6 per cent. solution of ether.



*Experiment XI.* Eggs from the oviduct were put into ether solutions of 1 and 0.5 per cent. for one-half and three-quarters of an hour; then washed in a small amount of fresh water and fertilized with the sperm from the vas deferens of the same individual. The experiment was carried out primarily in order to see if the eggs were affected by the solutions, so that they could be subsequently self-fertilized, but it is obvious that this test is not a good one, since the eggs will carry with them, despite the partial washing in water, some of the ether which may then act on the sperm. The results were as follows:

Ether	$\frac{1}{2}$ hour	$\frac{3}{4}$ hour
1.0	0	0
0.5	4	5

Without a check series, which unfortunately was not made, it is difficult to decide whether the small number of eggs that were self-fertilized was due to the action of the ether on the eggs or on the sperm. The experiment must be repeated on a more elaborate scale.

*Experiment XII.* In this experiment with two individuals, weaker solutions of ether were used. In one lot the sperm alone was put into ether, and then added to the eggs. In the other lot both eggs and sperm were put together into the ether. I omitted recording the time in the ether, but it was probably about five minutes.

Ether	Sperm and Eggs in Ether	Sperm only in Ether
1.0	0	0
0.5	0	2
0.25	0	0

Ether	Sperm and Eggs in Ether	Sperm only in Ether
1.0	20	0
0.5	50	0
0.25	85	4

This experiment shows that the sperm of the first individual was incapable of self-fertilization, even with the ether present. In the other individual, the sperm was good, and there was a

great deal of it; hence, no doubt, the excellent results in the first column. What is especially significant is that the best results were obtained when the eggs and the sperm were put at the same time into the solution together. This may mean the ether has some effect on the eggs as well as on the sperm, or that the most effective period of activity for the sperm is immediately after it comes into contact with the ether. My experiments do not suffice to settle this point, but that the spermatozoa are still capable of cross-fertilizing, after they have been in the ether for some time, is shown by the following result. After four hours the eggs of the first individual were mixed with the eggs and the sperm of the second individual. Later it was found that all the unsegmented eggs had been fertilized. The ether had no doubt largely evaporated.

The preceding twelve experiments with ether-solutions gave definite results, although in a few cases the number of eggs self-fertilized was small. It should be stated that there were ten other individuals in which self-fertilization in ether did not take place. This does not detract, I think, from the value of the successful experiments, because, as has been shown, the sperm is sometimes incapable of fertilizing even the eggs of another individual. The following experiments were carried out in order to examine this question further. It will be observed that parallel experiments with ether were also performed.

In each series five individuals were used. The eggs of each were fertilized with the sperm of every other individual. The following scheme shows the order in which the eggs were crossed. An individual having been opened, the eggs were removed from its oviduct and distributed in five dishes, A-A. Another individual was then opened (using, of course, different scissors, pipettes, etc.) and its eggs distributed to the next line of dishes, B-B. The same method was followed for the other three individuals. The sperm, a, of the first individual was then taken out and put into a small amount of water. It was then distributed to one set of eggs from each of the other individuals, B, C, D, E; then the sperm of B was taken out and applied to another set of eggs. The process was repeated until all the eggs were supplied with sperm. The

sperm in each case is indicated in the table by the small letter used as an exponent. The first set of A-eggs was as a rule fertilized with the e-sperm and the last set of E-eggs with the a-sperm.

*Experiment XIII.—*

E <sup>a</sup> 100	E <sup>b</sup> 100	E <sup>c</sup> 100	E <sup>d</sup> 100	E <sup>a</sup> 85
D <sup>a</sup> 100	D <sup>b</sup> 100	D <sup>c</sup> 100	D <sup>e</sup> 100	D <sup>e</sup> 100
C <sup>a</sup> 98	C <sup>b</sup> 100	C <sup>d</sup> 75	C <sup>d</sup> 75	C <sup>e</sup> 100
B <sup>a</sup> 99	B <sup>c</sup> 100	B <sup>c</sup> 100	B <sup>d</sup> 100	B <sup>e</sup> 100
A <sup>e</sup> (omitted to add sperm)	A <sup>b</sup> (omitted to add sperm)	A <sup>c</sup> 100	A <sup>d</sup> 85	A <sup>e</sup> 99

In this experiment practically all of the eggs were fertilized by the sperm of another individual. When fewer than the total number segmented (fertilized), immature eggs may have been present. As a check series A, B, C, D and E were self-fertilized. None segmented, except in E, where two eggs out of the twenty present, *i. e.*, 10 per cent. divided.

The two ether series (self-fertilized) of these same eggs gave the following results:

Ether	A	B	C	D	E
0.5	10	1	0	80	30
1.0	50	2	0	0	0

*Experiment XIV.* An experiment similar to the last was carried out, with five other individuals, and gave the following results:

E <sup>a</sup> 0	E <sup>b</sup> 0	E <sup>c</sup> 30	E <sup>d</sup> 100	E <sup>a</sup> 4
D <sup>a</sup> 0	D <sup>b</sup> 0	D <sup>c</sup> 0 (one egg)	D <sup>e</sup> (no ripe eggs)	D <sup>e</sup> 100
C <sup>a</sup> 0	C <sup>b</sup> 0	C <sup>d</sup> (no eggs)	C <sup>d</sup> 100	C <sup>e</sup> 100
B <sup>a</sup> 0	B <sup>c</sup> 0	B <sup>c</sup> 0	B <sup>d</sup> 50	B <sup>e</sup> 100
A <sup>e</sup> 0	A <sup>b</sup> 0	A <sup>c</sup> 0	A <sup>d</sup> 100	A <sup>e</sup> 100

Despite a few slight discrepancies in this table, the main result is clear. In only two of the five individuals was the sperm capable of cross-fertilization, namely, the e-sperm and the d-sperm.

There was also a self-fertilized series of these eggs, and in this not any of the eggs segmented. The ether series gave the following results:



Ether	A	B	C	D	E
0.5	0	0	0	0	10 (only ten eggs.)
1.0	0	0	?	2	50

It becomes evident from this result that the frequent failure of the sperm and eggs (mixed together) to self-fertilize in ether is due to the poor quality of the sperm. The poor sperm does not cross-fertilize, and presumably for the same cause it can not always be made to self-fertilize even in the ether. That sperm that is too poor to cross-fertilize may sometimes self-fertilize with the help of ether I hold to be possible. I regret that I did not attempt to determine whether poor sperm, that will not cross-fertilize, can be made to do so by means of ether, but other experiments lead me to think that it would often do so.

*Experiment XV.* In the following experiment all of the sperm appears to have been good except that of A, whose eggs, however, were in excellent condition.

E <sup>a</sup> 2	E <sup>b</sup> 85	E <sup>c</sup> 90	E <sup>d</sup> 0 (unfertilized)	E <sup>a</sup> 0
D <sup>a</sup> 1	D <sup>b</sup> 100	D <sup>c</sup> 20	D <sup>e</sup> 90	D <sup>e</sup> 100
C <sup>a</sup> 70	C <sup>b</sup> 100	C <sup>d</sup> 100	C <sup>d</sup> 100	C <sup>e</sup> 90
B <sup>a</sup> 5	B <sup>c</sup> 100	B <sup>c</sup> 100	B <sup>d</sup> 100	B <sup>e</sup> 100
A <sup>e</sup> 0	A <sup>b</sup> 70	A <sup>c</sup> 100	A <sup>d</sup> 100	A <sup>e</sup> 100

It is clear that the a-sperm was poor, although it did well in C<sup>a</sup> in which 70 per cent. of the eggs divided.

There was also a self-fertilized series in which none of the eggs segmented, except 5 per cent. in B. (In C, 90 per cent. of the eggs divided, but this may have been due to accidental contamination.) In the ether series the following results were obtained.

Ether	A	B	C	D	E
0.5	0	25	50	0	2
1.0	0	0	10	0	0

In this case although the spermatozoa of B, C, D, E were capable of crossing, they self-fertilized in ether very poorly, except in C, where good results followed.

*Experiment XVI.* In this experiment again only the first in-

dividual produced poor sperm, yet it did fairly well in one case, and in ether gave some results.

E <sup>a</sup> 5	E <sup>b</sup> 100	E <sup>c</sup> 80	E <sup>d</sup> 100	E <sup>a</sup> 70
D <sup>a</sup> 0	D <sup>b</sup> 100	D <sup>c</sup> 10 (few eggs)	D <sup>e</sup> 100	D <sup>e</sup> 30
C <sup>a</sup> 0	C <sup>b</sup> 100	C <sup>d</sup> 100	C <sup>d</sup> 100	C 100
B <sup>a</sup> 25 (only 4 eggs)	B <sup>c</sup> (no eggs)	B <sup>c</sup> (no eggs)	B <sup>c</sup> (no eggs)	B <sup>e</sup> (no eggs)
A <sup>e</sup> 75	A <sup>b</sup> 100	A <sup>c</sup> 100	A <sup>d</sup> 100	A <sup>e</sup> 100

In the self-fertilized series no eggs segmented. The ether series gave the following results:

Ether	A	B	C	D	E
0.5	2	4	2	30	0
1.0	50 (only 8 eggs)	12 (only 8 eggs)	0	10	30

The experiments recorded in Exp. XIII to XVI show that the sperm is at fault when cross-fertilization does not take place. In fact, eggs in the oviduct seem always to be capable of cross-fertilization. It is also evident that it is more difficult to get results with ether when the sperm does not cross-fertilize well, than when it does act well in this way. From this it seems to me very probable that when the ether fails to bring about self-fertilization the fault lies with the sperm. We may perhaps even go further and conclude that the action of the ether in bringing about the self-fertilization is on the sperm alone, but I am not in position to prove positively that the action of the ether on the eggs may not also enter into the result.

In concluding my account of these experiments on *Ciona*, I should like to point out that I had constantly in mind the possibility that the ether might produce parthenogenetic segmentation, and that the sperm had in reality nothing to do with the result. It was abundantly shown, however, that this was not the case, and in the few experiments in which I put this view to the test, by keeping eggs without sperm in ether-solutions of various strengths, I got no results when the eggs were returned to water. It should be noted in this connection that Lyon<sup>1</sup> has recently

<sup>1</sup> American Journal of Physiology, IX, July, 1903.

recorded that he was unable to cause artificial parthenogenesis in *Ciona intestinalis* at Naples by any of the ordinary means that excite this development in other eggs.

I shall discuss later the view as to whether eggs may be entered by the sperm of the same individual, but fail to develop unless incited to do so by some external agent.

It has been pointed out in the preceding pages that the eggs of *Ciona* may be fertilized after they have been in sea-water several hours. I made a test of this again in the following experiment:

*Experiment XVII.* Some eggs were cross-fertilized at once, others after 30, 80, 125 minutes, with fresh sperm from the same individual. All the eggs developed. A striking fact was observed in this case. The eggs fertilized late began to segment after a shorter interval than did those fertilized at once, so that at the 32-cell stage those fertilized last were only one division behind the first set, and no doubt soon caught up. It appears that a ripening process goes on in the egg as it stands in the sea-water, so that it begins to segment more quickly after it is fertilized than does an egg fertilized as soon as removed from the oviducts. It even appeared that after the first cleavage the rhythm of division was quicker in the eggs whose fertilization had been delayed, but this point needs a special examination which I have not yet made. The discovery is all the more significant because the first polar spindle is already formed in *Ciona* while the egg is in the oviduct, and the spindle remains resting in the equatorial plate stage until the egg is fertilized; hence the difference in time of segmentation can not be accounted for by the time required for the breaking down of the egg-nucleus and for the formation of the polar spindle after the egg has been removed from the animal. Some change must take place in the sea-water, which, while it does not cause the polar spindle to pursue its development, yet causes the developments that take place after the spermatozoön enters to go on more rapidly.

#### EXPERIMENTS WITH CYNTHIA

The ovaries of *Cynthia* extend far forward, and have a very short oviduct. Each ovary—there appear to be two in each in-



dividual—is double, the halves being united at the distal end. Owing to the close proximity of the ovary to the surrounding tubes of the testis, it is possible only by very careful manipulation to get the eggs out of the cavity of the ovary without cutting into the testicular tubes. When it was necessary to separate the eggs from the sperm of the same individual, I have carried out this operation, but in general the ovaries and the testes were cut up together.

For the purpose of studying the effects of self-fertilization Cynthia is in many respects inferior to Ciona because self-fertilization takes place to a very large extent. On the other hand, if check experiments are used for each individual, this factor can be estimated, and the very fact that Cynthia does self-fertilize its own eggs to such an extent gives an opportunity to examine other aspects of the problem. A much more serious difficulty is met with in that artificial cross-fertilization is often unsuccessful in this species. Even when the eggs and sperm from a large number of individuals are mixed together, fertilization may not take place; but in curious contrast to this result are the following observations on the egg-laying processes of this animal kept in aquaria. On several occasions a number of individuals were put together in the same dish. About 5 o'clock in the afternoon one after another began to send out jets of eggs and of sperm producing the effect of a lively cannonading. Under these circumstances it was found that every single egg was fertilized. Perhaps only ripe individuals sent out their eggs and sperm, or perhaps the eggs were mature in all individuals, and the sperm from one or two individuals may have sufficed to fertilize all of the eggs. In general it is, I think, the sperm of Cynthia that is not good. Certainly the spermatozoa are often very sluggish when taken from the testis and put into water. May it not be possible that when the eggs are laid, Cynthia secretes some other fluid that makes the sperm active? This point needs further investigation.

The best means that I found to determine the extent to which self-fertilization of the eggs of Cynthia may take place was to isolate some of the individuals early in the day, and observe in those that emitted eggs and sperm in the late afternoon the per-

centage of eggs that segmented. The following four records were obtained in this way: For August 11—33, 10, 100, 95, 95, 75, 10 per cent. For August 16—30, 30, 10, 1, 75, 90, 85 per cent. For August 19—33, 0, 10, 4, 4, 0. For August 20—12, 4. A much larger number of individuals gave off neither eggs nor sperm, and some produced sperm and no eggs, and *vice versa*. The results in the above list show all conditions from perfect self-fertility to absolute self-sterility, although some of the latter cases may have been due to no sperm being given off.

A few preliminary trials were made with two (A and B), and with three (A, B, and C) individuals. The scheme of crossing is given in the following diagrams:

For Two Individuals.

A <sup>a</sup>	B <sup>b</sup>
A <sup>b</sup>	B <sup>a</sup>

For Three Individuals.

A <sup>a</sup>	B <sup>b</sup>	C <sup>c</sup>
B <sup>a</sup>	A <sup>b</sup>	B <sup>c</sup>
C <sup>a</sup>	C <sup>b</sup>	A <sup>c</sup>

A few examples of the results with two individuals are as follows:

A <sup>a</sup> 0	B <sup>b</sup> 0	A <sup>a</sup> 0	B <sup>b</sup> 0
A <sup>b</sup> few	B <sup>a</sup> 15	A <sup>b</sup> 10	B <sup>a</sup> 10
A <sup>a</sup> 0	B <sup>b</sup> 0 (later rarely one)	A <sup>a</sup> 0	B <sup>b</sup> 0 (one egg)
A <sup>b</sup> few	B <sup>a</sup> 20	A <sup>b</sup> few	B <sup>a</sup> few
	A <sup>a</sup> 0	B <sup>b</sup> 0 (one egg)	
	A <sup>b</sup> 50	B <sup>a</sup> 0	

Comparing the self-fertilized eggs with the crossed-eggs, it is clear that while self-fertilization did not take place in nine cases, and in only one egg in the other case, yet cross-fertilization more frequently occurred, but never so completely as when many individuals normally deposited their eggs and sperm together. In addition to these cases there were three others in which none of the eggs, neither self- nor cross-fertilized, segmented. One of the results with three individuals is given in the next table:

A <sup>a</sup> 0	B <sup>b</sup> 2	C <sup>c</sup> 0
B <sup>a</sup> few	A <sup>b</sup> very few	B <sup>c</sup> 25
C <sup>a</sup> 75	C <sup>b</sup> rare	A <sup>c</sup> 4

In this experiment the a- and c-sperm did not self-fertilize, but the former did well with C- and the latter with B-eggs. The b-sperm self-fertilized to a slight extent, but did no better with the A- and with the C-eggs.

In the next series the results are more striking:

A <sup>a</sup> 0	B <sup>b</sup> 0	C <sup>c</sup> 0
B <sup>a</sup> 95	A <sup>b</sup> 50 ( <sup>rest not</sup> <sub>ripe</sub> )	B <sup>c</sup> 1
C <sup>a</sup> 50	C <sup>b</sup> very few	A <sup>c</sup> 50

Here none of the sperm self-fertilized the eggs. The a-sperm did quite well with the B- and C-eggs (95 and 50 per cent). The b-sperm did well with the A-eggs, but not with the C-eggs. The c-sperm did well with the A-eggs, but not with the B-eggs. It may appear from the preceding table that there is something more involved than simply the question of good sperm, for the same sperm appears to act differently with different eggs.

Another experiment with three individuals gave no eggs self-fertilized, but good cross-fertilizations with the c-sperm; less good with the b-sperm. These experiments should be carried out on a larger scale, and at different times of the year, but they suffice to show that self-fertilization is very infrequent when the process is an artificial one. It takes place to a considerable extent in some cases when eggs are normally laid. Moreover the artificially crossed eggs do not segment nearly so well in Cynthia as in Ciona.

The next experiment shows the action of ether on self- and cross-fertilized eggs. Some of the eggs and sperm of one individual, A, were removed and put into sea-water. Other eggs, A<sup>a</sup>, were self-fertilized in an ether-solution, and a third lot, A<sup>b</sup>, were crossed with sperm from B (A-sperm was also present). The same process was carried out with B which was crossed with sperm from A.

A 0	B 0
A <sup>a</sup> few	B <sup>b</sup> few
A <sup>b</sup> very few	B <sup>a</sup> very few

The results show that the self-fertilized eggs in ether did as well as those that were crossed, but none of the eggs in water alone,



with their own sperm, segmented. Another similar experiment with two other individuals gave the following results:

A	0	B	0
A <sup>a</sup>	0	B <sup>b</sup>	0
A <sup>b</sup>	50	B <sup>a</sup>	(only one egg 0)

In another set the ether appears to have been too strong, yet 50 per cent. of A<sup>b</sup> divided.

In another experiment, 10 per cent. of the self-fertilized eggs in ether segmented, and 50 per cent. of the crossed.

In another, 5 per cent. of the self-fertilized eggs in ether segmented, and 75 of the crossed.

The next set is more instructive:

A	0	B	0
A <sup>a</sup>	100	B <sup>b</sup>	0
A <sup>b</sup>	2	B <sup>a</sup>	4

It is clear that the ether had a marked effect in A<sup>a</sup>, making all of the eggs self-fertilize. This is all the more interesting because none of the eggs without ether self-fertilized. Both eggs and sperm of the B- set appear to have been in poor condition, so that the sperm did not cross-fertilize, or the eggs become cross-fertilized, to any extent.

In searching for other substances that might act on the spermatozoa as does the ether, I tried, amongst other things, a solution of ammonia in sea-water, and this I found made the spermatozoa even more active than the ether. Dilute solutions of alcohol from 1 to 10 per cent. also excite the spermatozoa to greater activity. Certain salt-solutions, ammonium chloride (1,  $\frac{1}{2}$ ,  $\frac{1}{4}$  per cent.), magnesium chloride (2 per cent.), and sodium chloride (1 per cent.) appeared also to act on the sperm, but much less effectively than does ether, alcohol, or ammonia. In the alcohol series of 1, 3, 5, 6, 10 per cent., it was found that 1 per cent. made the sperm very little more active; 3 per cent. more so; 5 per cent. most active; 6 per cent, less; 8 per cent, no effect; 10 per cent., no effect. The last two solutions undoubtedly injured the sperm. In another series, 7 per cent. gave the best results.

A few experiments were carried out in order to see if the sperm made active by the alcohol, would self-fertilize the eggs when it would not do so without the stimulus. Here, as in the preceding series, the same lettering will be used in the tables. A<sup>a</sup> self-fertilized in sea-water, A<sup>a</sup> self-fertilized in alcohol-solution, A<sup>b</sup> crossed in alcohol-solution.

A <sup>a</sup> 0	B <sup>b</sup> 0
A <sup>a</sup> [Alcohol] few	B <sup>b</sup> [Alcohol] several
A <sup>b</sup> 20	B <sup>a</sup> 50

In this experiment while no eggs were self-fertilized in sea-water, a few or several (the percentages were not recorded) were self-fertilized in alcohol, but even more developed in the crossed lots.

In another experiment only one individual was used. The eggs, self-fertilized in sea-water, did not segment, but 10 per cent. did so in a 3 per cent. solution of alcohol, and 50 per cent. in a 5 per cent. solution of alcohol.

Solutions of ammonia gave similar results. Sperm and eggs were mixed together in very dilute solutions of ammonia. Many eggs divided and of these most appeared, from their method of division into several cells at once, to be polyspermic. Some of the sperm from the last lot was added to eggs in sea-water. Fewer eggs were fertilized, but several that were fertilized were polyspermic. Eggs (not separated from their own sperm) were crossed in ether. All of these were polyspermic. Another set gave almost identical results.

It is clear from these experiments that those solutions that make the spermatozoa more active often induce fertilization of the eggs, when such a fertilization does not take place without the use of the solutions. The activity of the sperm and the fertilization of the egg appear to be directly connected. This point will be more fully discussed later.

#### EXPERIMENTS WITH MOLGULA.

On each side of the body of Molgula there is an ovary surrounded by a testis. It is very easy to open the central cavity of the ovary, and remove the eggs without cutting the testis.

A few preliminary experiments showed that the sperm of *Molgula* fertilizes the eggs of the same individual. The following illustrations will show the great powers of self-fertilization of this species:

A <sup>a</sup> 85	B <sup>b</sup> 90	A <sup>a</sup> 100	B <sup>b</sup> 2
A <sup>b</sup> 90	B <sup>a</sup> 100	A <sup>b</sup> 100	B <sup>a</sup> 90 [irregular
A <sup>a</sup> 100	B <sup>b</sup> 100	A <sup>a</sup> 0	B <sup>b</sup> 100
A <sup>b</sup> 100	B <sup>a</sup> 100	A <sup>b</sup> few	B <sup>a</sup> 0
	A <sup>a</sup> -90		B <sup>b</sup> 0 <sup>1</sup>
	A <sup>b</sup> 100		B <sup>a</sup> 100

These cases make it clear that the sperm is capable of fertilizing the eggs of the same individual. Whether the sperm of another individual is prepotent I did not attempt to determine. There were only a few cases in which neither self- nor cross-fertilization was effective, and whenever good crossing was accomplished self-fertilization was also realized, showing that when the sperm is good, it will readily fertilize the eggs of the same individual. Since similar results were obtained when three individuals were used it will not be necessary to give the latter cases. The experiments were not extensive enough to show whether good sperm affects the eggs of certain individuals better than it does others, but *Molgula* is not well suited to test this point.

It occurred to me as possible that in *Cynthia* and in *Molgula* the power to self-fertilize the eggs might be due to the eggs coming from the ovary on one side of the body, and the sperm from the other side. Conversely, if this were true, the lack of self-fertilization in *Ciona* might be connected with the presence of only one ovo-testis. I examined this possibility for *Molgula*. The eggs from the small ovo-testis were fertilized with sperm from the same side, and other eggs with the sperm from the other side. In both cases all the eggs were fertilized. Conversely, the eggs from the large ovary were fertilized with sperm from the

<sup>1</sup> In B the sperm was probably bad. The A<sup>b</sup> must therefore have been self-fertilized. The same conditions hold also for the second couple.



same side, and others with sperm from the opposite side. Here also all the eggs segmented. It is perfectly evident, therefore, that the question of self-fertilization in *Molgula* is not connected with the double condition of the ovo-testis.

#### EXPERIMENTS WITH OTHER FORMS.

In order to find out how generally ether, alcohol, and ammonia excite to greater activity the movements of cilia, of flagella, and of the spermatozoa of other animals, I made a few experiments on certain protozoa and on the spermatozoa of the frog and of the rat.

Ether, 5 per cent. stops the movements of paramoecium, and kills stentor; 3 per cent. slows up the movements of the former, and causes stentor to throw off its outer layer; the movements of free swimming vorticellae seemed to be increased; 2 and 1 per cent. hasten the movements of paramoecium and of stentor.

Alcohol of 6 and of 8 per cent. slow down the movements of paramoecium and stylonychia, and cause stentor to disintegrate; 10 per cent. kills; 4 per cent. appears to be near the limit, and seems to increase their activity; 2 per cent. clearly increases their activity.

Ammonia 1/200 per cent. kills paramoecium, stentor, and stylonychia; and even 1/2000 also kills; 1/5000 per cent. seems to make these protozoa somewhat more active, but I have not sufficiently tested this solution.

Some of the same solutions were used with euglena, which moves by means of an anteriorly directed flagellum. Ether 5 per cent. makes them somewhat more active; 3 per cent. less so, and 2 per cent. gives no very noticeable effect. Alcohol 10 per cent. kills; 8, 6, and 4 per cent. make them swim more actively; 2 and 1 per cent. give no definite result. Ammonia 1/200 kills; 1/2000 per cent. does not appear to make euglena more active, but other strengths should be tried.

A male spotted frog (*Rana halcina*) was killed in November; its testes opened, and the immobile sperm squeezed out into normal salt-solution. It was found that it took some minutes to get a noticeable effect. Ether 5 and 2

per cent. caused the spermatozoa to show some movement in the course of 15 minutes. Alcohol gave better results. A 10 and a 6 per cent. solution awakened the spermatozoa to activity; a 4 per cent. gave the best results of all. In no case, however, was the activity very great. No movements were detected in ammonia-solutions, but only two strengths were used.

These scattering and incomplete observations show that these substances are in all probability general stimulants for protoplasmic activity of certain kinds.

I have also made a few experiments with the spermatozoa of mice. The spermatozoa were taken directly from the testis of a mouse that had just been killed. The solutions were added to a drop of the sperm squeezed out from the testis into a drop of physiological salt-solution, consequently the dilution is greater than actually given by the percentage. In certain strengths of ether (5 per cent.) and of alcohol (8 per cent.) it appeared that the movement was increased; with ammonia I did not get satisfactory results. The observations are made more uncertain here because, when the testes are opened, spermatozoa in all stages of development are found, and are consequently acted upon differently by the solutions. It would be more satisfactory to use a larger animal and take the spermatozoa from the vasa deferentia, where they are all fully formed. It is certain, however, that alcohol and ether do not produce as great effects on these spermatozoa as they do on the spermatozoa of the ascidians and of some other marine animals that I have examined.

In one of the preparations of the mouse testis the water began to run out at one side and it became apparent at once that the spermatozoa all turned and headed up-stream. It has been recorded by Kraft that spermatozoa swim in the opposite direction to that in which the cilia of the oviducts act. My observation suggests that movement in this direction is not due to the spermatozoa swimming against the direction of the greatest action of the cilia, but against the stream that is produced by the cilia. The movement may be a simple physical phenomenon—the lighter tails of the spermatozoa being swept backwards by the current so that the heads are turned up-stream, and the contraction of the tail then causes the spermatozoön to travel in this direction.

In later experiments the sperm was taken from the vasa deferentia, and put first into distilled water where the spermatozoa remained quiescent. If a drop of salt-solution (water 100, NaCl 0.75) was added to a drop of water containing the spermatozoa, they became active in the course of a minute or less, and their activity continued to increase for several minutes longer, when they remained active for some time. If a drop of a 5 per cent. ether solution is added to a drop of water containing quiescent spermatozoa, no result is seen at first, but after ten minutes I have observed a slight vibration of the spermatozoa. If now after the ether has been added, a drop of the salt-solution is also added, the spermatozoa become active, but it is difficult to determine whether they become more active than when the salt-solution alone is present. Certainly there is no marked difference. If a drop of 8 per cent. alcohol is added to a drop of water containing the spermatozoa no activity is observable, but if then a drop of salt-solution is also added the spermatozoa begin to swim, showing that the alcohol had not injured them, although it had failed to arouse them to activity. Several strengths of KOH (3 per cent. and weaker) were tried, but without effect; yet if salt-solution was added later some slight activity was seen.

In another series of experiments the spermatozoa quiescent in water were first made active by adding the salt solution. If ether was then added no decided effect on the sperm could be seen when their activity was compared with that of check preparations of salt-solution only. It appeared sometimes as though the ether did make the activity more pronounced, and the movement of the spermatozoa appeared somewhat different in the two cases. In the ether the motion was more jerky, and in the salt solution more sinuous and normal.

The following solutions were also tried: The sperm was first put into a drop of water, and then a drop of the solution was added.  $\text{NaHCO}_3$  0.625 per cent. caused the sperm to vibrate rather actively;  $\text{Na}_2\text{CO}_3$ , 5.0 per cent. caused a little activity after five minutes; KCl, 0.5 per cent. caused greater activity than did the sodium carbonate, while CaCl caused somewhat less vibration.



These, and some other experiments that need not be described here, show that salt-solutions of various kinds have a marked effect in arousing to activity the inactive spermatozoa of the vasa deferentia. They also make active, spermatozoa that are quiescent in distilled water. On the other hand ether, alcohol, and ammonia, which proved so efficient for the spermatozoa of the sea-urchin and starfish, appear to have little effect on the spermatozoa of the mouse.

The more fundamental physiological question as to the nature of the action of these different substances I shall not attempt to discuss without a further basis of observation and experiment to go upon. Enough has been seen, however, to suggest that the substances act as a "stimulus," which is perhaps not dissimilar in kind from that which causes some eggs to begin to develop, or a nerve impulse to start, or a muscle to contract. Here also we may urge, as I have urged elsewhere<sup>1</sup> in opposition to Loeb's conclusion in regard to the action of certain agents in causing artificial parthenogenesis, that the nature of the stimulus is of such a kind that the result depends much more on the structure or the composition of the living thing than upon the kind of stimulus employed. So unstable is the living organization that the slightest change brought about in it by chemical or by physical means suffices to set into action a perfectly definite and pre-arranged series of events.

#### HISTORICAL REVIEW.

The action of ether, ammonia and alcohol on the spermatzoa of *Ciona*, arousing them to greater activity and thus, under certain conditions, bringing about the fertilization of the egg, raises the question as to whether in the higher animals a similar action may not result from the application of these and of other substances, and also whether the secretions of some of the glands connected with the reproductive system may not have a similar effect on the spermatozoa.

When I tried to find some substances that might bring about self-fertilization in *Ciona* I was not aware that there had already

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<sup>1</sup> Science. N. S. XI. 1900. Pp. 178-180.

been made several experiments on the action of solutions on the spermatozoa of other animals. I find that there are quite a number of observations of this sort, although none of the observers have had in view the same question with which I was especially concerned.

Kölliker in 1856 carried out an extensive series of experiments on the effect of different solutions on the spermatozoa of the bull, dog, rabbit, horse, and also made a few observations on the spermatozoa from a human cadaver. He found that water alone quickly brings spermatozoa to rest, but does not kill them. They can be aroused to activity by adding, for instance, a 10 per cent. solution of disodium phosphate.<sup>1</sup> Many other substances were found favorable to the activity of the spermatozoa, such as blood-serum, sugar in certain strengths, sodium chloride, caustic potash, etc.

The caustic alkalies (potassium, sodium, and ammonium hydroxide) were found to be especially powerful excitants. Kölliker also tried a number of other solutions, such as three different kinds of sugar, glycerine, gum, etc., which in certain strengths cause increased activity; also urea, gall, morphine, strychnine (nitricum), which have an indifferent effect. He also tried alcohol, creosote, chloroform, ether, alkaloids, and tannin, which have an injurious effect. Kölliker also examined the action of the secretions of the glands of the male reproductive organs—the uterus masculinus, prostate and Cowper's glands. He found that these secretions excite the spermatozoa to greater activity.<sup>2</sup>

The much more recent experiments of Steinach bear even more directly on the present problem. He found that after removal of the glandulae vesiculares ("receptaculum seminalis" of some writers) of the male rat, that, although the sexual instinct remained, the number of young that were born was much decreased. When

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<sup>1</sup> Kölliker gives the formula  $2\text{NaOH}\cdot\text{H}_2\text{PO}_4$ , which is no doubt disodium phosphate, now written  $\text{Na}_2\text{HPO}_4$ .

<sup>2</sup> Moleschott and Richetti are quoted by Kölliker as recognizing the favorable action of sodium salts on the spermatozoa. Quatrefages found that the spermatozoa of the weasel showed a "surexcitation" in 64 parts sea water to one part sea salt. Newport found that potassium carbonate, and also 1/480 of potassium salt made the activity of the spermatozoa of the frog greater.

this gland, as well as the prostate, was removed no young at all were born, although frequent union with the females took place. The results may be due to the semen being insufficiently diluted when it is not mixed with the secretions of the glands, or else to the absence of proper excitation of the spermatozoa when the gland-secretion is removed. That the spermatozoa may be normally acted upon by the secretion of the glands was shown by Steinach in the following way: Sperm from the vas deferens was mixed with a physiological salt-solution. A drop was placed under a cover slip and the edges sealed to prevent evaporation. The preparation was kept at a temperature of  $35^{\circ}$  to  $37^{\circ}$  C. A similar preparation was made with the secretion of the prostate. In the former the spermatozoa began to lose their activity in one and a half hours, and after three hours had come completely to rest. In the other preparation, that containing the extract from the prostate gland, the spermatozoa were active after 11 hours, and ceased to move altogether only after 22 hours. This experiment shows that the secretion of the gland prolongs greatly the period of activity of the spermatozoa. Whether it excites them to greater activity is not stated, but Köl liker's results leave no doubt on this score. The decrease in the fertilizing power when the glands were removed may well be connected, as suggested above, with the lessened activity of the spermatozoa.

Buller has recently studied the question as to whether the spermatozoa of the sea urchin are attracted to the egg,—in other words, whether, as some authors have assumed off-hand to be the case, there is a chemotactic action of the egg on the spermatozoa. He points out that although Strasburger claimed that the egg of *Fucus* excretes a substance that attracts the spermatozoön from a distance of two diameters of the egg, Bordet and Buller himself have failed to confirm this statement. Massart thinks that in the case of the frog the meeting of the spermatozoön and the egg is purely accidental. Buller finds for the sea-urchins, *Arbacia*, *Echinus*, and others, that when the spermatozoa are set free near the egg they show no tendency to swim towards it. The dense collection of spermatozoa that forms around the egg is due to those that happened to run into the jelly sticking there. These



spermatozoa then proceed to bore into the jelly; most of them in a radial direction, although a few can be seen to go in obliquely, or tangentially. The same phenomenon occurs in unripe eggs, and in eggs that have been killed in weak osmic acid and the acid washed out. It is improbable, therefore, that chemotaxis has anything to do with the result.

In order to see if any substance is given off by the eggs that attracts the spermatozoön, the eggs were taken from the ovary, carefully washed, and allowed to stand for 2 to 12 hours in a small amount of sea-water. Capillary tubes were then filled with this water and placed in a drop containing spermatozoa. The spermatozoa did not show any tendency to collect around the openings of the tubes. Several other substances were tried in the tubes in the same way,—salts, sugar, ferments, acids, alcohol, etc.—but no chemotaxis was discovered.

The spermatozoa of the sea-urchins swim in spirals. Coming into contact with a surface, the spiral is changed to a circular movement due to contact. Buller considers whether the radial path taken by most of the spermatozoa after they have entered the jelly is due to stereotropism. He reaches the conclusion that while theoretically this assumption will explain the phenomenon, yet conclusive evidence in favour of this view is lacking. He suggests that it may be possible to find a purely physical solution of the problem.

Von Dungern has examined the question of cross-fertilization from the point of view of the different substances contained in the egg, and has reached some conclusions of the greatest interest. He finds that the egg of the starfish, *Asterias glacialis*, contains a substance that acts as a poison on the sperm of the sea-urchin (*Echinus* or *Sphaerechinus*). The minimal lethal dose for the sperm mixed with 2 ccm of sea-water varies considerably with the individual; for *Echinus* between 1/800 to 1/6400 part is fatal in half an hour. Von Dungern tried to obtain an antitoxin from the blood of the rabbit that would neutralize the effect of the poison of the eggs, hoping that it might be possible in this way to bring about the cross-fertilization of the egg of the starfish by the spermatozoön of the sea-urchin. He found, however, that

the serum of the normal rabbit already contains a substance that has a powerful antitoxic action on the poison of the starfish, so that it was not necessary to obtain an antitoxin by injecting the poison into the rabbit. The antitoxin of the rabbit's serum was added to water containing the eggs of *Asterias*, and then sperm from a sea-urchin was supplied. Von Dungern often obtained two- and four-cell stages in this way, but the results were uncertain, and he could not decide whether fertilization had or had not taken place. It seems not improbable, I think, that the outcome may have been due to artificial parthenogenesis which occurs very readily in the eggs of certain starfish; in fact, it is very difficult to prevent its occurrence, unless the eggs are very carefully handled.

The same poison that is present in the eggs of the starfish is also secreted by the skin. It is also rendered harmless by the rabbit's serum. In the sea-urchin there is a poisonous substance in the gemmiform pedicellariae, which is very injurious to the sperm of the starfish. If 100 of the pedicellariae of *Sphaerechinus* are rubbed up in one ccm of sea-water, the solution will destroy in a quarter of an hour the sperm contained in ten to twenty litres of sea-water. The minimal lethal dose for 2 ccm is 1/5120 to 1/16240 ccm. The spermatozoa of *Sphaerechinus* itself are killed by this fluid, but a much stronger dose is necessary. On the other hand an extract of the egg of *Echinus*, *Sphaerechinus*, *Strongylocentrotus*, or *Arbacia* does not kill the spermatozoa of the starfish even in the strongest solutions. What then prevents the spermatozoa of the starfish from entering the eggs of these sea-urchins? There is another factor, Von Dungern thinks, that interferes with this combination. The egg membrane of these urchins has an agglutinizing effect on the spermatozoa of the starfish. This agglutinizing effect appears to be the same phenomenon as that seen "whenever cells of any kind are introduced into the body of another animal." So far as this process is involved in the union of germ-cells, Von Dungern thinks that under certain conditions it might assist the fusion, while under others it might interfere with it. Thus two naked and equivalent cells might be helped to unite, while an egg surrounded by an agglu-

tinizing jelly would fail to be fertilized. The substance in the sea-urchin's egg that agglutinizes the starfish sperm can be rendered ineffective by the rabbit's serum. Not all starfish spermatozoa are agglutinated by the jelly or by the egg-substance of all the different sea-urchins. In *Sphaerechinus* it fails to occur. Therefore in this case the failure to cross-fertilize must be due to some other factor, and, in fact, Von Dungern claims to have found still another substance in the sea-urchin's egg that excites to greater activity the immature and quiescent spermatozoa of the starfish. These immature sperm, made active by this substance, are then capable of fertilizing the eggs of the starfish. He found that weak doses of chloral hydrate and of cocaine also make these quiescent spermatozoa active, and that rabbit's serum has a marked effect. Von Dungern believes further that these exciting substances may actually prevent, in certain cases, the cross-fertilization, because they may change the kind of reaction shown by the sperm. He observed that the spermatozoa of those species that do not normally show rotational movements when they come in contact with surfaces, usually do so when the exciting substances just mentioned are present. It does not appear to me, however, that this is an altogether satisfactory explanation of the failure of cross-fertilization in these cases.

Von Dungern also examined the question as to whether the egg secretes a substance that favours fertilization by its own sperm. He believes that he has also discovered such a substance. The eggs of *Echinus* (or of *Sphaerechinus*) are rubbed up and mixed with pieces of jelly that have been carefully washed. When sperm is added to the water in which such pieces lie they stand vertically to the surfaces of the pieces. If on the other hand the pieces of jelly are not mixed with the substance from the egg, the spermatozoa simply rotate on the surface of the jelly, and do not stand vertically. Starfish spermatozoa with *Arbacia* jelly behave as with simple jelly alone, *i. e.*, they do not stand vertically. The vertical position of the spermatozoa is due, Von Dungern thinks, to the presence of some substance in the extract that lowers the excitability of the spermatozoön to contact, and hence it takes a vertical position. He also points out that this same substance



causes the spermatozoa to lose their power of movement in a short time. Thus, while Von Dungern finds no evidence of a substance in the egg that attracts the sperm, he believes that there may be present in some eggs a substance that favours the fertilization of the egg, by causing the spermatozoon to assume that position in the jelly that is most likely to bring them to the surface of the egg.

Loew has attempted to show by an experiment, which is not, I think, well suited to prove his point, that the spermatozoa of the rat are attracted to, *i. e.*, that they are positively chemotactic to, the slime layer of the uterus and also to the alkaline mucosa of the digestive tract, but not to the acid slime of the vagina. His method of experimenting was as follows: A piece of the mucosa of the uterus was put on one side of a slide and a piece of the vagina on the other. A drop containing the sperm was placed in the middle of a cover-slip, and this put over the pieces on the slide. It was found that the sperm collected more on the side near the piece of the uterus, and from this Loew infers that they have been attracted to this side. In the light of the other experiments described above it will be clear, I think, that the greater accumulation of the sperm on one side by no means establishes the conclusion that they have been attracted to this side. Loew tried to show that filter paper saturated with alkaline substances acts chemotactically on the spermatozoa, in the sense that they move towards such substances, but, as in the preceding case, it does not necessarily follow because spermatozoa collect around or in certain substances, therefore they must have moved towards these substances. The recent work of Jennings on the protozoans shows that their accumulations in certain areas is not due to the action of substances that cause the individuals to swim towards those substances, but on the contrary to their action being such that those individuals that enter areas containing these substances are unable to leave them. The *result* is the same as when the spermatozoa touch the jelly of the egg and stick to it, although the *means* by which the accumulations are formed in the two cases are entirely different. It would be interesting to see if spermatozoa may not behave towards certain solutions as do the protozoans.

THEORETICAL.

It has been often assumed by embryologists that there exists some sort of attraction between the eggs and the spermatozoa of the same species. This idea would readily suggest itself to anyone who saw spermatozoa collecting in crowds around the eggs, but it by no means follows that this phenomenon is really due to an attracting substance emanating from the egg. The result may be due to the membrane of the egg, to which those spermatozoa stick that come accidentally into contact with it. In fact I have observed similar collections of spermatozoa in the ascidian around pieces of the body tissue, where the result had every appearance of being due to some sticky substance, exuding from the piece, rather than to an attraction exerted by the piece on the spermatozoa.

Pfeffer's oft-quoted experiment with the antherozoöids of ferns, liverworts, etc., appears to support the idea that the antherozoöids are attracted to the malic acid that is present in the neck of the archegonia, but in the light of the recent experiments of Jennings and others, as to the way in which unicellular forms accumulate in a drop of acid, we can readily see that the results may have a very different interpretation from that usually given to them. Confining our discussion to the results obtained with the ascidians, I offer the following tentative analysis of the problem:

The failure of the spermatozoön of *Ciona* to enter the egg of the same individual may be conceived as due to some physical obstacle. It is conceivable that pores may exist in the egg-membrane, or even in the surface of the egg itself. This is the argument that Pflüger<sup>1</sup> used in the case of cross-fertilization of the frog's egg. If in the ascidian there existed a correlation of such a sort, that the size of a spermatozoön of a given individual is always greater than the pores of the eggs of the same individual, then self-fertilization could not take place. That this is not the real explanation is shown by the fact that good spermatozoa are apparently capable of fertilizing the eggs of all other individuals. This would certainly not be the case if the exclusion of the sperma-

<sup>1</sup> Archiv. f. die gesammte Physiologie, XXIX., 1882.

tozoön from the egg of the same individual was due to the size of the pores, because there would be eggs of some other individuals having pores as small or smaller. Another possibility that suggests itself is that the surface tension of the egg is of such a sort that it excludes the spermatozoa of the same individual, but this idea does not appear to give a satisfactory solution, for, aside from the fact that it is difficult to imagine how such a relation could exist, there would also occur cases in which the surface tension of the eggs of other individuals would exclude certain sperm, and this does not appear to be the case. It is true that the addition of the ether to the water may cause a difference in the surface tension of the egg, and it might be made to appear that this was the way in which the self-fertilization is effected in the ether-solutions, but I can not believe that this is the explanation of the results, because other experiments show that a considerable amount of ether is necessary to cause self-fertilization.

It seemed to me that violent shaking might so affect the surface of the egg that self-fertilization might take place. A number of eggs from the oviduct were violently shaken for a few minutes in a small vial, and then sperm from the same individual was added. No segmentation took place, and the presumption is therefore that the eggs were not fertilized.

Turning to the chemical side we find a number of possibilities that demand consideration. The inactivity of the immature spermatozoa, and the lack of power of such sperm to fertilize the egg, their becoming active in certain solutions, and their power then to fertilize eggs that they did not fertilize before, as best shown in *Cynthia*, suggests that normally the eggs may secrete certain substances that make more active the spermatozoa, which then become capable of fertilizing the eggs. This view appears all the more attractive in the present case on account of the observed lethargy of the spermatozoa of these ascidians, and the apparent connection in such cases between this condition and the impotence of such sperm in fertilization. Yet after careful consideration I am not prepared to advocate this view as the only solution, although I realize that it might be made to give the appearance of a ready explanation of my results. Not that this induced activity may not



be one of the factors to be taken into consideration, but it is not, I think, the whole explanation. My reasons for regarding this view as insufficient are the following: It was found that sperm that appeared to be very little active was sometimes capable of cross-fertilizing the eggs of another individual. Possibly this may be due to somewhat greater activity induced by something secreted by the eggs of the other individual, yet on the whole I can not claim that direct observation gave any convincing evidence in favour of this assumption. More significant are the results of the experiment of mixing eggs from two individuals, and subsequently fertilizing them with the sperm from one of the individuals. Half only of the eggs segmented, presumably those cross-fertilized. If some substance that makes the sperm active were really thrown out by the eggs, then we should expect that all the eggs would have been fertilized, unless indeed the secretion loses its power a short distance from the surface of the egg that secretes it; but this does not seem to be a probable interpretation.

A different point of view is that the egg secretes some substance that attracts the spermatozoa. On this view we must suppose that the substance secreted by the egg of *Ciona* has no attraction for the spermatozoa of the same individual.

The little evidence that I have to offer, based on experiments with ascidians, is not favorable to this idea, that the cross-fertilization is due to some attractive substance secreted by the egg. In the species that I have examined there is no such marked accumulation of spermatozoa around the eggs as is seen in many other animals, and nothing in the behaviour of the cross- and self-fertilized egg to suggest that the difference in the results is due to an attraction in the one case, and to the absence of an attraction in the other. In other forms where there is a better opportunity for examining this question the most recent observations go to show, as has been pointed out in detail above, that there is no sufficient evidence for the view that the egg attracts the spermatozoön.

Conversely, it may be supposed that the egg secretes some substance that *repels* the spermatozoa of the same individual. I

observed nothing that would support such a conclusion, and this interpretation of the process would be foreign to what we find in general in connection with fertilization even in cases where the sperm of one species does not fertilize the eggs of another.

We come now to a more subtle argument, and one that we are scarcely in position to discuss profitably in our present state of ignorance concerning the union of egg and spermatozoön. It may be assumed that there is some sort of "chemical affinity" between the egg and the spermatozoön that causes the two to unite when they come together. On this assumption we should have to suppose in *Ciona* that this affinity does not exist, or at least is less strong, between the egg and the spermatozoa of the same individual than between those of different individuals. Such a statement carries us no further, however, than the facts, and in the case of *Cynthia* we should have to assume that the affinity is so nicely balanced that sometimes the spermatozoön can unite, and sometimes it can not. In the case of *Molgula* the affinity must be assumed to suffice to bring about self-fertilization. Until we can give some more tangible form to this idea it does not appear to have any greater value, than the mere statement of the facts, and indeed may have less value, since it may give a wrong impression as to the real factors at work.

Finally there might be advanced what may be called the electro-chemical hypothesis. The union of the egg and the spermatozoön may be supposed to be an electrical phenomenon, connected with a difference in the chemical composition of the two elements. The sperm head is almost pure nuclear chromatin, while the surface of the egg is protoplasmic. Possibly the spermatozoön and the egg have different electrical charges and unite with each other if brought near enough for the charges to become effective. But on this supposition it is not clear why the eggs and the sperm of the same individual would not unite. Here also we get no light on the absence of self-fertilization in *Ciona*.

I have kept constantly in mind while at work on this problem the possibility that the spermatozoön may really enter the egg, but fail to develop there, or fail to start the development of the egg, because, coming from the same individual, it was not sufficiently

different in composition to supply the necessary stimulus. The ether might be supposed to make the sperm sufficiently different from the egg to start the cleavage, or the ether might itself supply the stimulus which is capable of starting the development of the egg *after* the spermatozoön has entered.

The test of this view should be found in direct observation of the eggs themselves. I prepared therefore a series of eggs of *Ciona*, some unfertilized for check series, others self-fertilized, but not put into ether, and others like the last, but put into ether.

The difficulties of determining whether the spermatozoa can enter the eggs of the same individual, but fail to start the development, are greater than may appear at first sight. The sperm head is so minute that if after it entered no changes were affected in the protoplasm about it, its presence might be readily overlooked, and since the spermatozoön of *Ciona* enters the egg in a granular zone that colors more deeply in certain stains than does the rest of the egg, the difficulty is thereby increased. Of course I have been on my guard against cases where the surrounding sperm have floated over the section, as sometimes happens, or have been carried over it by a defect in the knife, and I have also been careful to exclude all cases where specks of foreign matter may have been on the slide, or in the fixative. There are also two further precautions to be taken. When the egg withdraws from the membrane and the test-cells are extruded, as it were, from the outer zone of the egg, the protoplasm is sometimes drawn out in mamiliform processes that stain deeply and resemble the entrance cone formed by the spermatozoön penetrating certain eggs. Even when the protoplasm does not protrude, deeply staining spots are generally present and are especially obvious after iron haematoxylin. Careful staining with Delafield's haematoxylin shows clearly that these spots have nothing to do with the entrance of spermatozoa. Furthermore these spots are found in unfertilized eggs. After iron haematoxylin minute deeply staining bodies, flattened against the outer surface of the egg, can generally be found, and these strongly suggest spermatozoa. That they are not such is shown by their presence in unfertilized eggs, and by their absence after the Delafield



stain. I mention these points because they might easily lead one after only a casual examination to conclude that spermatozoa enter the eggs. My best results have been obtained by drawing out the iron haematoxylin until the protoplasm has lost all of its color, or better still by using the Delafield stain, and also thoroughly extracting the color from the protoplasm.

Although I have examined a large number of preparations I have not seen a single definite case without ether in which a spermatozoön has entered the egg of the same individual. Difficult as it admittedly is to be absolutely certain on this point, yet if the spermatozoa had entered and had begun to enlarge I feel certain that I should have detected their presence. That undeveloped sperm-heads may be present I must admit as a possibility, but I have not detected them, and believe that I should have been able to do so were they present. It is also a point of some importance that I have not found any spermatozoa within the egg membrane, although quantities of them may lie outside.

There is a further point in this connection, the importance of which I did not appreciate until I had closed the experimental part of my work. In the eggs of many animals a change takes place in the egg, after the penetration of one spermatozoön, of such a sort that the entrance of more spermatozoa is prevented. I have found in *Ciona* that, after the sperm has stood with the eggs of the same individual and has failed to fertilize them, these eggs could still be readily fertilized by spermatozoa from another individual. If a spermatozoön of the same individual really enters the egg it does not in consequence bring about such a change in the egg that other spermatozoa can not enter, and therefore many spermatozoa of the same individual from which the eggs were taken should be expected to gain entrance, but I am quite certain that this, at least, does not occur. From this consideration also it may be inferred that the spermatozoa do not normally penetrate the eggs of the same individual.

In the light of these observations it seems probable that whenever a spermatozoön enters the egg, the egg begins to develop regardless of whether the spermatozoön comes from the same or from another individual. The ether must therefore induce a change

of some sort that directly effects the entrance of the spermatozoön into the egg, and at present I see no other interpretation that is left than that this entrance is due to the greater activity of the spermatozoön that causes it to overcome some resistance, either on the surface of the egg itself, or in the membrane surrounding it. The nature of this resistance I did not detect, and this must be the next step in the analysis. One method by which this view may be tested is obvious, and has already been referred to. The spermatozoa made active by sea-water must be placed in an extract of the eggs (or body-tissues) of the *same* individual, and then, after a time, the eggs of another individual added. On the hypothesis these eggs *should be less likely* to become fertilized than eggs placed directly in contact with the fresh sperm.

It has been found that certain substances secreted by the glands of the reproductive organs of the male mammal arouse the spermatozoa to greater activity. It has also been found that many other substances have a similar effect on spermatozoa. It would be equally interesting to discover if the secretions of other parts of the genital ducts of the male or of the receptacula of the female, when such are present, may not bring the spermatozoa to rest, or keep them quiescent until some other exciting agent arouses them. It seems almost certain that this must be the case in those animals in which the spermatozoa of the male are stored up in receptacula of the female, as for instance in the honey bee, or in such a hermaphroditic animal as the earthworm. The length of life of the spermatozoa in some of these forms would seem to make some assumption of this sort necessary. Experiments can easily be made that would decide this question. Kölliker has shown, in fact, that water quiets the spermatozoa of mammals without killing them.

In the ascidians it is probable that the spermatozoa in the vas deferens are quiescent. It is significant that in these hermaphroditic forms the oviduct in which the eggs are stored takes a course parallel to the male duct. Possibly the proximity of the two ducts may be connected with the lack of power of self-fertilization of the eggs, because the egg may be saturated with the same substances that keep the sperm quiescent. It may be, however,

that this relation is more fundamental, and the particular substance is one peculiar to the whole body. That the reaction must be something quite specific is shown by the fact that the spermatozoa are able to enter eggs of any other individual.

It appears probable that of all the different substances that excite the spermatozoa to activity the secretions of the glands connected with the male reproductive organs may be the most efficient. From a statement of Kölliker's it seems not improbable that the substance secreted in the glands of one species may be also efficient for the spermatozoa of other species. Whether by the use of the substances from the glands of another mammal it might not be possible to excite human spermatozoa to greater activity and thus assist materially in bringing about fertilization in cases where the impotence is on the side of the male remains to be examined. There is here a question that may have an important practical aspect.

The lack of power to self-fertilize in plants may also be due to the inability of the pollen tube to penetrate sufficiently far into the stigma and style. It appears that penetration does actually begin in some cases that have been observed, but possibly the growth may be arrested further down in the style. The prepotency of other pollen would then find its explanation in the more rapid growth of this foreign pollen. Here again is an opportunity for future work.<sup>1</sup>

In attempting to formulate a theory to account for the determination of sex, Castle assumes that there are two kinds of spermatozoa, male and female, and that there are also two kinds of eggs, male and female. He also assumes that a female egg can be fertilized only by a male spermatozoön and that a male egg only by a female spermatozoön. I have already pointed out elsewhere<sup>2</sup> that my results do not support this assumption. Castle appealed to the case of *Ciona* as one in favour of his contention, for the eggs here can not be fertilized by the sperm of the same individual. It is not explicitly stated to the contrary, and the reader might be led to infer from the context that in *Ciona* all the eggs and all

<sup>1</sup> The experiments of Myoshi should be especially considered.

<sup>2</sup> Popular Science Monthly, Dec., 1903.



the spermatozoa of one individual must be supposed to be male, and in another individual the reverse; but certainly this is not the case, and could not have been Castle's meaning, for if it were so then half of the individuals would be infertile with the sperm of the other half, and this is not so. I have pointed out that my results with ether, etc., do not support Castle's assumption, although it might, of course, be claimed that the ether causes the spermatozoa to lose, as it were, their homosexual repugnance. However this may be, I have found that no such lack of power to self-fertilize is found in some other ascidians, as in *Molgula* for example. If my supposition is correct, that self-fertilization in *Ciona* is due to the presence in the eggs of some substance that brings the spermatozoa to rest, the whole question assumes a very different aspect and does not appear to have any connection with the question of the determination of sex.

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# THE INFLUENCE OF CALCIUM AND BARIUM ON THE SECRETORY ACTIVITY OF THE KIDNEY.<sup>1</sup>

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In previous publications<sup>2</sup> it was shown that subcutaneous or intravenous injections of small quantities of solutions of certain salts, including the saline purgatives, produce not only increased peristalsis, but also an increased secretion of fluid into the intestine. This was found to be true also when the solutions were applied locally to the peritoneal surfaces of the intestine. It was suggested that the main actions of saline purgatives consist in the production of increased peristaltic movements, and of increased secretion of fluid into the intestine; and that the semi-fluid faeces which are produced by saline purgatives are the result not of decreased power of absorption by the intestine, but of an increased secretion of fluid into the intestine. It was further shown that the administration of calcium or magnesium chloride tends to suppress the peristaltic movements and the secretory activity of the intestine. Attention was specially called to the marked action of barium chloride in the production of violent peristaltic movements and ringlike constrictions in the intestine, and also in the production of an increased flow of fluid into the intestine. It was also pointed out that the production of these activities in the intestine by the purgative salts, and their suppression by calcium and magnesium is analogous to the production and suppression of rhythmical contractions in voluntary muscles de-

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<sup>1</sup> A preliminary report of these experiments was published in the University of California Publications, Physiology, January 15, 1904, Vol. I., No. 10, p. 81.

<sup>2</sup> MACCALLUM, J. B.—American Journal of Physiology, Vol. X., No. III., p. 101, 1903, and Vol. X, No. V, p. 259, 1904.



scribed by Loeb<sup>1</sup>. The antagonism which has been shown by Loeb to exist between the actions of many sodium salts on the one hand, and calcium and magnesium salts on the other was further illustrated by these experiments.

It seemed possible then in the light of these facts that the activities of the kidney might be controlled in the same way as those of the intestine. Since it is well known that many sodium salts have a distinct diuretic action, it seemed conceivable that calcium or magnesium might act as an antidiuretic. In order to decide this point I have made a series of experiments in which I have found that the relation of many of the salts to the activity of the kidneys is similar to that which they bear to the glandular activity of the intestine.

#### METHODS.

The following experiments were carried out mainly on rabbits; a few dogs also were used. In all cases morphine was given as an anaesthetic. The rabbits received 3-5 cc. 1% solution of morphine hydrochlorate subcutaneously; the dogs in addition to this dose of morphine were given ether when necessary.

The urine was collected by catheterising the ureters or by tying a cannula in the bladder. The latter method was employed in all cases except those in which it was necessary to observe the difference between the amounts secreted by the two kidneys. In placing a cannula in the bladder a small incision was made in the abdominal wall. The bladder, which usually contains a considerable quantity of urine, was then lifted out of the body cavity, and the abdominal wall sewed up around the neck of the bladder so that the intestines could not be forced out. A purse-string suture was then made in the fundus of the bladder and an incision made in the bladder wall within the suture. In this way the urine could be removed, and the cannula securely tied in. Care was taken to allow no urine to collect in the bladder, so that the measurements given in the tables represent all the urine that was secreted during each period. The simple catheterisation of the bladder through the urethra may be easily performed in rabbits,

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<sup>1</sup> LOEB, J.—Festschrift für Fick, 1899; *Archiv. für die gesammte Physiologie*, 1902, XCI, p. 248.

but this method is unsatisfactory when it is necessary to obtain the exact amount of urine secreted in a given time since it is impossible to tell whether the bladder is at any time entirely empty.

Solutions of the salts whose actions were tested were introduced into the body intravenously. In rabbits a hypodermic needle was placed in a vein of the ear; in dogs the fluid was forced into one of the superficial veins of the lower limb. When small quantities were injected a hypodermic syringe was used; when larger amounts were introduced a pressure apparatus was employed. This was the apparatus commonly used in injection work, consisting of a pressure bottle connected on one side with a water tap, and on the other with a graduated bottle containing the solution to be injected. In this way a constant pressure could be obtained, and the quantity of fluid injected in a unit of time accurately controlled. By causing the fluid to pass through a coil of rubber tubing immersed in hot water before reaching the needle, the solution could be kept constantly at the body temperature. Some of the details of the apparatus were suggested to me by an apparatus used by Dr. M. H. Fischer in this laboratory. For such infusions into the blood only  $m/8$  solutions were employed; in subcutaneous injections stronger solutions were used. Except in those cases where it was necessary to obtain the effect of the salt on the normal flow of urine, the secretion was considerably raised and kept constant by the uniform infusion of  $m/8$  NaCl solution throughout the experiment. The effect of the other salts was then obtained by allowing small quantities of  $m/8$  solutions to flow into the vein along with the NaCl solution. In other instances these salts were injected into a vein of one ear while the NaCl solution was at the same time flowing into the opposite ear. In these experiments the ear of the rabbit was securely tacked to the board, and the needle kept from slipping out of the vein by means of bull-dog forceps.

#### EXPERIMENTS.

The results of the experiments on the actions of calcium and barium may be best seen in the following tables:

1. Dog—small terrier—cannula placed in right ureter.  
Urine secreted in 1st 10 minutes..... 3.6 cc.  
2d 10 minutes..... 3.6 "

8 cc  $\frac{m}{8}$   $\text{CaCl}_2$  injected into vein of leg.

Urine secreted in 1st 10 minutes.....	2.4 cc
2d 10 minutes.....	2.2 "
3d 10 minutes.....	1.8 "
4th 10 minutes.....	1.6 "
5th 10 minutes.....	1.4 "

10 cc  $\frac{m}{2}$  sodium citrate injected subcutaneously.

Urine secreted in 1st 10 minutes.....	1.6 "
2d 10 minutes.....	2.3 "
3d 10 minutes.....	3.1 "
4th 10 minutes.....	3.6 "

In this case the secretion of urine gradually decreased after the injection of calcium chloride until the amount collected in a unit of time was less than half of the initial amount. The addition of sodium citrate to the blood counteracted this effect so that the rate of secretion again approached the normal. These effects are more striking when the quantity of urine secreted is increased by the introduction of normal salt solution into the blood as shown in the following experiment:

2. Rabbit—cannula placed in bladder. No urine flowed in the first or second periods of 10 minutes before the  $\text{NaCl}$  solution was injected.

Time.	Salts other than $\text{NaCl}$ injected.	$\frac{m}{8}$ $\text{NaCl}$ injected in cc.	Urine in cc.
10.10	—	10	—
10.15	—	10	—
10.20	—	5	0.5
10.40	—	10	0.8
11.00	—	10	0.5
11.20	—	5	1.0
11.40	—	10	2.8
12.00	—	10	6.0
12.00	5 cc $\frac{m}{8}$ $\text{CaCl}_2$ intravenously		
12.05	5 cc $\frac{5m}{8}$ $\text{CaCl}_2$ subcutaneously		
12.20	—	5	0.2
12.40	—	10	1.8
1.00	—	10	0.8
1.00	5 cc $\frac{m}{8}$ sodium citrate intravenously		
1.20	—	10	2.2
1.40	—	5	3.6



In this experiment, although the flow of urine has been considerably increased by the injection of  $\frac{m}{8}$  NaCl, the introduction of  $\text{CaCl}_2$  markedly suppresses the secretion. The flow of urine remains small for an hour, although a somewhat greater quantity of fluid is forced into the blood than in the previous hour. This suppression of urine is at once counteracted by the injection of sodium citrate.

The following table (3) which represents only the latter half of an experiment shows roughly the duration of the action of smaller doses of calcium.

3. Rabbit—cannula in bladder—injections intravenous.

Time.	Salts other than NaCl injected.	$\frac{m}{8}$ NaCl in- jected in cc.	Urine in cc.
9.25	—	—	—
11.40	—	150	64.5
11.45	—	10	6.6
11.50	—	10	5.6
11.55	—	10	6.2
12.00	—	10	7.4
12.05	—	10	9.5
12.05	5 cc $\frac{m}{8}$ $\text{CaCl}_2$	—	—
12.10	—	5	2.2
12.15	—	10	0.8
12.20	—	10	1.2
12.25	—	10	1.6
12.30	—	10	2.8
12.35	—	8	3.0
12.40	—	5	4.5
12.45	—	0	4.8
12.50	—	0	5.1
12.55	—	0	6.2

As shown here and in other experiments, the action of calcium is only temporary. I have found also that magnesium chloride in many cases has an antidiuretic action similar to that of calcium chloride. The suppression of urine, however, is not so marked as with calcium.

As shown in the following experiment (4) barium chloride in very small doses has a strong diuretic action. Although it is much more powerful in this respect than sodium citrate, the increased flow of urine which it causes may be suppressed by the injection of calcium chloride.

## 4. Rabbit; injections intravenous.

Time.	Salts other than NaCl injected.	$\frac{m}{8}$ NaCl injected in cc.	Urine in cc.
10.20	—	—	—
10.30	—	20	—
10.40	—	10	—
10.50	—	20	1.2
11.00	—	32	2.8
11.10	—	28	5.8
11.20	—	20	6.1
11.30	—	10	8.2
11.40	—	10	8.3
11.40	$\frac{1}{8}$ cc $\frac{m}{8}$ BaCl <sub>2</sub>		
11.50	—	10	14.4
12.00	—	10	18.0
12.10	—	10	12.4
12.10	$\frac{1}{8}$ cc $\frac{m}{8}$ BaCl <sub>2</sub>		
12.20	—	10	18.4
12.30	—	10	16.4
12.30	5 cc $\frac{m}{8}$ CaCl <sub>2</sub>		
12.40	—	10	8.6
12.50	—	10	4.0
1.00	—	10	2.0
1.10	—	10	2.4
1.20	—	5	3.4
1.20	$\frac{1}{4}$ cc $\frac{m}{8}$ BaCl <sub>2</sub>		
1.30	—	8	6.4
1.40	—	10	8.2
1.50	—	10	8.6
1.50	$\frac{3}{4}$ cc $\frac{m}{8}$ BaCl <sub>2</sub>		
1.55	—	10	1.8
2.00	—	10	0.6
2.10	—	10	0.0
2.20	—	—	0.0
2.30	—	—	0.0

In the uniform injection of considerable quantities of normal salt solution into the blood, the flow of urine, after about an hour, becomes fairly constant. If an average amount of 1 cc. in 1 minute be introduced, the secretion of urine during the first two or three hours is usually slightly less than the amount of fluid injected. After this time, when no other salts have been added, the quantity injected and the quantity secreted may become

approximately equal. As shown in experiment 4 however the addition of a minute quantity of  $\text{BaCl}_2$  (less than  $\frac{1}{2}$  cc  $\text{m}/_8$  solution) to the blood causes the flow of urine to increase markedly, so that the quantity secreted in a unit of time is far in excess of the quantity of fluid introduced into the blood. If, however, while this active secretion is going on, 5 cc.  $\text{m}/_8$   $\text{CaCl}_2$  solution be injected into the blood, the flow of urine rapidly decreases, although the total quantity of fluid added to the blood remains constant. The further addition of  $\text{BaCl}_2$  again increases the secretory activity so that the quantity secreted in 10 minute periods which has fallen from 16.4 to 2, under the influence of  $\text{CaCl}_2$  is again raised to 8.6 by the injection of the barium salt. An apparently contradictory thing, however, happens when a larger amount of barium chloride is suddenly added to the blood. As shown in the foregoing table, while  $\frac{1}{8}$  cc.  $\text{BaCl}_2$  largely increases the urinary secretion, the injection of  $\frac{3}{4}$  cc. in addition to that already present, causes an entire cessation of the flow of urine. In some cases this suppression of the flow of urine is quite abrupt; in other instances it is more gradual, a few drops of urine flowing from the cannula at intervals. As shown in the following experiment, the injection of  $\text{CaCl}_2$  sometimes counteracts this action of larger doses of  $\text{BaCl}_2$  and causes the urine to flow again.

5. Rabbit—cannula in bladder; injections intravenous.

Time.	Salts other than $\text{NaCl}$ injected.	$\text{m}/_8$ $\text{NaCl}$ in- jected in cc.	Urine in cc.
9.55	—	—	—
10.00	—	10	1.0
10.15	—	15	3.4
10.30	—	15	5.2
10.45	—	15	5.1
11.00	—	15	4.8
11.00	1 cc $\text{m}/_8$ $\text{BaCl}_2$ + 4 cc $\text{m}/_8$ $\text{NaCl}$	15	4.8
11.15	—	10	2.4
11.30	—	15	0.2
11.45	—	15	0.3
11.45	5 cc. $\text{m}/_8$ $\text{CaCl}_2$	15	0.3
12.00	—	10	2.0
12.15	—	15	3.8
12.30	—	15	4.0



In this case 1 cc  $\frac{m}{s}$   $BaCl_2$  gradually suppresses the flow of urine, and no trace of the strong diuretic action of barium is seen. And, further, calcium chloride has here an action which seems at first glance entirely opposed to that which it ordinarily has. As shown in the previous experiments, calcium characteristically suppresses the secretion of urine. In this case the flow of urine increases after its administration. These apparent contradictions may be explained in the following way. In discussing the actions of calcium and barium on the intestine, it was pointed out that barium chloride, like the other saline purgatives, affects the intestine in two ways, namely, by increasing the peristaltic movements and by increasing the secretion of fluid into the lumen. Attention was further called to the violent character of the muscular contractions in the intestine caused by barium, which may so constrict the lumen of the intestine that fluid cannot pass from one part to another. It was also shown that calcium to some extent counteracts the action of barium both on the muscle, and on the glands of the intestine. It seems therefore probable that the increase in the flow of urine caused by small doses of barium chloride ( $\frac{1}{8}$  cc.  $\frac{m}{s}$  solution) is due to an increase in the secretory activity of the kidney entirely analogous to that which is produced in the intestine by the same salt. The cessation of the flow of urine however which follows the administration of larger doses of barium chloride (1 cc.  $\frac{m}{s}$  solution) is in all probability due to the action of the barium on the muscle coats of the urinary passages, especially those of the calyces and pelvis of the kidney, and those of the ureter. Since all of these various parts of the urinary passages are surrounded by thick, circular and longitudinal muscle coats, not unlike those of the intestine, it seems conceivable that a strong contraction of these coats, such as barium is capable of causing in the intestine might effectually shut off the lumen so that no urine could pass. Furthermore the action of calcium in renewing the flow of urine under these circumstances is quite analogous to its action in suppressing the peristaltic movements or in relieving the constrictions in the intestine caused by barium. The actions of calcium and barium which are shown in Table 5, are on the muscle coats of the urinary passages. It is quite conceiv-

able however that this action of calcium may coexist with its characteristic action in diminishing the secretory activity of the kidney. In both the intestine and the urinary apparatus (kidney, and urinary passages) barium stimulates the glandular and the muscular tissues to activity. Calcium on the other hand uniformly suppresses these activities.

It must be pointed out however that the suppression of the flow of urine which follows a relatively large dose of barium chloride cannot always be relieved by calcium. As was found to be true in the intestine, the action of barium is seldom completely counteracted by calcium. In many cases the barium stops the flow of urine entirely so that it is not possible to start it again. This is shown in the following experiment (6) where relatively large quantities of calcium chloride are incapable of reestablishing the flow of urine. This naturally suggests the idea that the large doses of barium may stop the secretion of urine by injuring the cells of the kidney, or perhaps indirectly by a constricting influence on the blood vessels. These possibilities must be taken into consideration; but the fact that calcium sometimes causes the urine to flow again after it has been inhibited by barium speaks strongly in favor of the theory advanced above, that the inhibiting action of barium on the flow of urine is an action on the muscular tissue of the urinary passages.

6. Rabbit—cannula in bladder; injections intravenous.

Time.	Salts other than NaCl injected.	$\frac{m}{8}$ NaCl in- jected in cc.	Urine in cc.
9.50	—	—	—
10.20	—	23	—
10.30	—	20	—
10.40	—	25	0.8
10.50	—	28	1.8
11.00	—	20	2.8
11.10	—	16	4.6
11.20	—	10	5.4
11.30	—	10	5.8
11.40	—	12	5.2
11.50	—	15	7.2
11.51	$\frac{2}{3}$ cc $\frac{m}{8}$ BaCl <sub>2</sub>	—	—
11.55	—	—	1.0

12.00	————	15	14.4
12.00	$\frac{1}{3}$ cc $\frac{m}{8}$ BaCl <sub>2</sub>	————	
12.05	————		1.6
12.10	————	14	0.0
12.20	————	12	0.0
12.30	————	12	0.0
12.32	5 cc $\frac{m}{8}$ CaCl <sub>2</sub>		
12.40	————	15	0.0
12.50	5 cc $\frac{m}{8}$ CaCl <sub>2</sub>	13	0.0
1.00	————	10	0.0
1.10	————	15	0.0
1.20	————	20	0.0

It will be noticed in this experiment (6) that immediately after the injection of  $\frac{2}{3}$  cc.  $\frac{m}{8}$  BaCl<sub>2</sub> solution there is a marked diminution in the flow of urine followed within a few minutes by a very considerable increase. This partial cessation of the flow immediately following the injection is due probably to a temporary action of the barium on the muscle coats of the urinary passages. The subsequent increase is the result of the diuretic action of barium on the kidney as described above.

In considering the actions of calcium and barium we must therefore take into account not only their influence on the glandular tissue, but also their effect on the muscular tissue of the body. In all cases these salts are antagonistic in their action; and their influence on the secretory activity of the kidney and on the flow of urine is entirely analogous to their influence on the glandular and muscular activities of the intestine. With regard to its action on the kidney calcium chloride may be properly termed an *anti-diuretic*.

Attention must be again called to the extremely poisonous nature of barium chloride. A subcutaneous injection of 3 cc  $\frac{m}{8}$  BaCl<sub>2</sub> solution is usually sufficient to kill a rabbit. Intravenously it should always be injected with four or five times its volume of  $\frac{m}{8}$  NaCl solution.

#### CONCLUSIONS.

1. In dogs and rabbits the quantity of urine secreted in a unit of time may for a time be markedly diminished and in some cases almost entirely inhibited by the introduction of calcium chloride into the circulation.



2. Calcium chloride diminishes not only the normal flow of urine, but also that which is caused by the administration of saline diuretics. For example, the rate of secretion which has been largely increased by the intravenous injection of normal salt solution may be temporarily lessened to a marked extent by the introduction of  $\text{CaCl}_2$  into the blood.

3. In all cases  $\text{m}/_8$  solutions were used, and  $\text{m}/_8$   $\text{NaCl}$  solution was introduced into the blood at a constant rate throughout the experiments. After a short time the rate of secretion became constant. It was then found in rabbits, that the addition of a small quantity of  $\text{BaCl}_2$  ( $1/8$  cc  $\text{m}/_8$  solution) to the blood causes a marked increase in the flow of urine, so that the amount of fluid secreted may considerably exceed that which is introduced into the blood during the same period of time.

4. This action of barium is counteracted by the injection of  $\text{CaCl}_2$ .

5. If a larger quantity of  $\text{BaCl}_2$  (1 cc  $\text{m}/_8$  solution) be added to the blood, the flow of urine ceases and often complete anuria ensues. In some cases the injection of  $\text{CaCl}_2$  abolishes this inhibitory action so that the urine flows again. Usually however the action of barium persists.

6. The fact that barium when given in smaller and in larger doses may thus apparently have opposite effects on the flow of urine may be explained by analogy with its action on the intestine. Barium chloride causes not only an increase in the secretion of fluid into the intestine, but also active peristaltic movements, and violent local constrictions of the intestine. Similarly very small doses of  $\text{BaCl}_2$  increase the secretory activity of the kidney. It seems probable however that the cessation of the flow of urine which follows the injection of larger quantities of the salt is due not to an inhibition of secretion, but to the action of the barium on the muscular coats of the urinary passages, especially those of the calyces and pelvis of the kidney and those of the ureter. This action would bring about a constriction of the tubes and a closure of the lumen. The fact that calcium counteracts both effects of the barium supports this explanation.

7. The influence of calcium and barium on the flow of urine is in every way analogous to their action on the intestine, which I have previously described. The suppression of the urinary secretion by calcium is also analogous to the suppression of twitchings in voluntary muscles by calcium, which has been described by Loeb.

In conclusion it is a pleasure to thank Professor Loeb for the interest which he has taken in these experiments. I am indebted also to Dr. Theo. C. Burnett, who has assisted me in many of the experiments.

# THE INHIBITIVE ACTION OF THE ROENTGEN RAYS ON REGENERATION IN PLANARIANS.

BY

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The great capacity of regeneration possessed by fresh-water planarians is well known because of the number of investigations lately devoted to the subject.<sup>1</sup> Complicated portions of the body, like the head and pharynx, when removed, are restored within a few days or weeks. In several species a new individual may be regenerated from a very small, isolated piece. We have found that this power of regeneration may be completely destroyed by exposing planarians to the action of the Roentgen rays.

Our experiments have been conducted upon two species of planarians which have especial regenerative capacity, *P. maculata* and *P. lugubris*. Specimens were placed in shallow, open dishes about ten to fifteen centimeters below the vacuum tube and were exposed from ten to twenty minutes each day for varying periods of time. We made use of a twenty-centimeter coil with an interrupter of the electrolytic type, a ten-inch coil with a mechanical interrupter, and several styles of vacuum tubes. The vacuum of each tube was so arranged that rays of "medium-soft" quality were obtained.

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<sup>1</sup> In addition to the literature quoted in Morgan's "Regeneration," New York, 1901, articles have appeared as follows: F. R. LILLIE, *American Journal of Physiology*, VI., p. 129, 1901; E. SCHULTZ, *Zeitschrift f. wissenschaftliche Zoologie*, LXXII, p. 1, 1902; N. M. STEVENS, *Archiv. f. Entwicklungsmechanik*, XIII., p. 396, 1901; T. H. MORGAN, *Archiv. f. Entwicklungsmechanik*, XIII., p. 179, 1901; *Biological Bulletin* III., p. 132, 1902; H. F. THACHER, *American Naturalist*, XXXVI., p. 633, 1902; C. R. BARDEEN, *Biological Bulletin* III., 262, 1902; *Archiv. f. Entwicklungsmechanik*, XVI., p. 1, 1903.



Our first experiments were upon worms from each of which the anterior region of the body was removed immediately before the first exposure. The cut edges became closed in by muscular contraction and the extension of epithelium in the usual manner. For some days there was a slight production of new tissue near the cut surfaces, but this soon ceased. No new heads were produced and no new pharynges. In one specimen of *P. maculata*, however, an imperfect eye was regenerated on the left side at the junction of the old tissue with the new, and a very small eye-spot appeared on the right side, but the anterior end of the piece at no time assumed the normal shape of a head. The specimens were subjected to thirteen exposures. All died between the twentieth and twenty-second days after the first exposure. Control specimens regenerated in the usual manner. A "tail-piece" of *P. maculata* had become a worm of perfect form and proportions on the fifteenth day after the operation, and a "tail-piece" of *P. lugubris* had regenerated a well proportioned new head and a new pharynx at that time.

Several experiments were made to test the effect of the rays on uninjured worms. Specimens were exposed from twelve to eighteen times and were then kept under as hygienic conditions as possible. Some of these individuals lived a month after the first exposure. During this period they reacted normally to light, to mechanical and to chemical (food) stimuli. Microscopical preparations made from a few specimens at varying periods after the last exposure showed no marked alterations in the muscular, nervous and intestinal apparatus. The cutaneous epithelium seemed to be normal except for a few areas where the cells were shorter and broader than usual. The cilia of the ciliated cells were intact. In specimens with well-developed genitalia the cells of the testes showed no karyokinesis. On the contrary most of them seemed to be undergoing a degenerative change. In corresponding control specimens mitosis was most active in these cells. No clear instances of nuclear degeneration were observed. Death in these specimens resulted from a degenerative process which began in the region of the head and extended slowly back. This degeneration was probably parasitic

in nature and seemed due possibly to insufficient protection offered by the thinned epithelium. Young individuals, of which we had several hatched out from an egg-capsule of *P. lugubris*, a few weeks before the experiments began, were affected like the mature specimens, but more quickly.

From worms exposed from ten to fifteen times to the rays pieces were cut immediately in some instances, and in some instances a week or ten days after the last exposure. In each instance the cut surfaces were closed by muscular contraction and mechanical extension of the surface epithelium, but in no instance was there subsequently seen any sign of the production of new tissue at the cut surface or in a region of the body where under normal conditions a new pharynx would be formed. Microscopical sections of a specimen killed within the second twenty-four hours after the isolating cut was made showed no signs whatever of cell-division, either direct or indirect. In control specimens mitosis was most active in the tissue-forming parenchymal cells at this period. In the exposed specimens the epithelium where it had extended out to cover a cut surface remained a flat, thin membrane as long as the specimen lived. In the control specimens it was quickly restored to its normal columnar form. The exposed individuals lived for from twenty-five to thirty days from the time of the first exposure. One piece of *P. maculata*, from which the head had been removed, lived for forty-one days. Reactions to light and to mechanical and chemical stimuli seemed normal in all the specimens.

From these experiments it is evident that the Roentgen rays have a powerful inhibitive effect upon cell-reproduction in planarians. It may be entirely stopped by sufficient exposure. No effect was noticed in the physiological activities or in histological structure of the highly differentiated tissues such as those of the nervous system and the musculature. The effects of the rays do not appear for some days after the first exposure. Thus there is a slight production of regenerative material at a cut surface in a specimen sectioned before exposure to the rays. The subsequent differentiation of an imperfect eye in one specimen indicates that the rays have effect not so much upon tissue differ-

entiation as upon cell-reproduction. The spreading out of the surface epithelium so as to cover a cut surface, whereby columnar epithelium becomes transformed into pavement epithelium also indicates this. Death in exposed specimens may possibly be due to a necessity on the part of the organism for a certain amount of cell-reproduction.

The effects of the Roentgen rays on planarians thus tend to support the view of those investigators who regard its effects upon the tissues of other animals as due primarily to its action on cells capable of reproductive activity. Scholtz in his excellent clinical and experimental studies on the effects of X rays on the mammalian skin<sup>1</sup> concludes that both the nuclei and the cell-protoplasm of the epithelial cells are injured by the rays, but that the effect on the connective tissues, elastic tissue, musculature and cartilage is very slight if any. The skin on both sides of a rabbit's ear may be affected when it is exposed to rays on one side only.

The effect is not, however, a direct one upon the actual process of cell-division. This is shown in planarians by the production of tissue at a cut surface during the first few days of exposure to the rays. It is indicated also by the work of Gilman and Baetjer on chick embryos<sup>2</sup> which showed that exposed hen's eggs develop even faster than control eggs for a few days although subsequently development is markedly altered and checked. One of us, likewise, found that exposure to Roentgen rays repeated frequently throughout the day for several days failed to prevent the normal course of development in the eggs of certain sea-urchins and teleosts during the period of exposure. The latent period between exposure to the rays and the development of a burn is well known to clinicians.

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<sup>1</sup> "Ueber den Einfluss der Roentgenstrahlen auf die Haut in gesunden und kranken Zustände," *Archiv f. Dermatologie und Syphilis*, LIX, pp. 87, 241, 421, 1902.

<sup>2</sup> Some effects of the Roentgen rays on the development of embryos, *American Journal of Physiology*, X, p. 222, 1904.



While the effect of the Roentgen rays is seen chiefly in the inhibition or alteration of reproductive activity in the cells of animal tissues it is improbable that it is limited to the results of such action. Schaudinn has shown<sup>1</sup> that individuals of several species of protozoa may be killed by exposure to the Roentgen rays for a few hours. Other forms are not, however, thus susceptible.<sup>2</sup>

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<sup>1</sup> Archiv f. die gesammte Physiologie, LXXVII, p. 29, 1899.

<sup>2</sup> Schwarz in a recent interesting paper (Über die Wirkung der Radiumstrahlen, Archiv. f. die gesammte Physiologie C, 532, 1903) concludes that the action of radium rays is due to a decomposition similar to that of a dry distillation brought about in albumenoid bodies of the cell. He explains their effect on rapidly growing tissues as due to their special power to decompose lecithin.



# EXPERIMENTAL STUDIES IN GERMINAL LOCALIZATION.

EDMUND B. WILSON.

## II. EXPERIMENTS ON THE CLEAVAGE-MOSAIC IN PATELLA AND DENTALIUM.

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The first of these studies (*Journ. Exp. Zoölogy*, I, 1, 1904) was especially concerned with the question of cytoplasmic pre-localization in the unsegmented molluscan egg, and gave only an incidental account of experiments on the cleavage. In that paper both cytological and experimental evidence was presented to show that the *Dentalium* egg contains from the beginning definitely specified regions, consisting of visibly different materials, which stand in such a relation to the morphogenic process that the removal of particular areas of the unsegmented egg produces corresponding definite defects in the resulting larva. It was shown, further, that during the cleavage process these materials are definitely distributed to the blastomeres of the early embryo, and that when these blastomeres are isolated they give rise always to defective larvæ, showing the same general character as those derived from the corresponding regions of the unsegmented egg. I therefore concluded that the development of these eggs sustains His's theory of germinal prelocalization ("Organbildende Keimbezirke") as applied to the unsegmented egg, and Roux's mosaic theory as applied to the cleavage process, and is in harmony with the theory of formative stuffs.

In that paper, the evidence for the mosaic character of the cleavage was given only in part, including only a brief account of the general development, in *Dentalium*, of isolated blastomeres from the 2-cell and 4-cell embryos, and of isolated micromeres of the first quartet. The present paper offers more detailed evidence in the same direction, derived mainly from experiments on *Patella carulea*. The comparative ease and certainty with which blastomeres of any desired stage may be obtained by means of Herbst's calcium-free sea-water led me to hope that a fairly complete experimental analysis of the potencies of the cleavage cells might be carried out; and I do not doubt that in time such an analysis can be effected. Various practical difficulties, however, have rendered the analysis here offered incomplete in several directions. Nevertheless the positive results attained form the most detailed and, as I think, convincing evidence of mosaic development thus far produced, and in my judgment clearly demonstrate this general principle in the molluscan egg. Despite the

obvious gaps that they show in some directions, I therefore publish the results as they stand, with the hope that they may be extended hereafter.<sup>1</sup>

#### METHODS.

The eggs of *Patella carulea* were obtained in a mature state from March until June, those of *Dentalium entalis* during June and July. Artificial fertilization is easily effected in *Dentalium*, but is much more difficult in *Patella*. In the latter case I found, after many trials, that the eggs fertilized more readily if first placed for half an hour in sea-water rendered slightly alkaline by the addition of 4-6 drops of a 5% solution of potassium or sodium hydrate to half a litre of sea-water (the slight precipitate first formed quickly dissolves upon agitation). The spermatozoa were also placed in the alkalized water for the same length of time. From 15 to 20 minutes after fertilization (in the same water) the eggs were as a rule transferred to a large quantity of pure sea-water brought from the open sea.

In both forms the opaque egg is at first surrounded by a very distinct membrane, which, in the case of the ripe eggs, disappears as the eggs lie in water before fertilization, in *Patella* by gradually dissolving away and disintegrating at several points, in *Dentalium* by suddenly bursting and being thrown off. Double fertilization occurs rarely in *Dentalium*, but very frequently in *Patella*, so that in the latter case it is essential to pick out the normal eggs one by one with a pipette at the 2-cell stage. In both forms the blastomeres can be separated with the greatest ease by means of Herbst's calcium-free sea-water—indeed, the action is so energetic that better results are obtained if it is restrained somewhat by mixing the artificial water with a certain amount of normal sea-water. The eggs were placed in the artificial water shortly after both polar bodies had formed, and after division the blastomeres were carefully separated under the lens with a fine scalpel and immediately isolated in normal sea-water. Even so, however, the blastomeres often continue to separate in the normal water, and the best results for the earlier stages were obtained by not employing the artificial water, but by separating the cells with the scalpel in normal water. This is difficult in *Patella*, but very easy in *Dentalium*, in the earlier stages. For somewhat later stages the artificial water must be used; but this can often be successfully accomplished by transferring the 2-cell stages to normal water and separating the blastomeres at the proper stage. The tendency to separate after transference to normal water steadily decreases as the development proceeds; hence good results for still later stages are obtained by allowing the eggs to segment in the artificial water up to the 16-32-64-cell stages, before isolation and transfer to normal water. For greater certainty of identification the best plan is to separate and isolate the blasto-

<sup>1</sup>Like the preceding work, this was done at the Naples Zoölogical Station between February and the end of July, 1903, on a grant from the Carnegie Institution of Washington. I would again express my great indebtedness to the administration of the Station for the unremitting care and efficiency with which my work was aided.

meres after each division, transferring them to normal water at the stage desired and all my critical results have been thus attained. The mortality is very large, since the blastomeres seem to suffer severely in the change from the artificial to the normal water, and is greatest in cells from the vegetable hemisphere; hence my failure thus far to isolate successfully the second somatoblast (or primary mesoblast-cell, 4d), in some respects the most interesting of all the cells. For the latest stages I did not endeavor to isolate the cells at all, but allowed the eggs to develop for 24 hours in the artificial water, from time to time separating the cells by jets from a fine pipette.

Most of the studies on isolated blastomeres were made on *Patella*, since with *Dentalium* most of my time was given to experiments on egg-fragments. For preparation of the *Patella* eggs I found no better method than the simple one employed by Patten ('85) of acetic acid and glycerine. The eggs were placed in a watch-glass nearly filled with sea-water, two to four drops of glacial acetic acid added, followed by successive additions of dilute glycerine gradually replaced with strong glycerine. This renders the embryos perfectly transparent, with sharply marked cell-boundaries, and often gives preparations of admirable clearness. A slight stain with acetic carmine often adds considerably to the effectiveness of the preparation for a time, though they subsequently deteriorate, and for most purposes the stain is superfluous.

## II.

### PRELIMINARY NOTES ON THE NORMAL DEVELOPMENT.

Unfortunately the cell-lineage of neither *Patella* nor *Dentalium* has been worked out. Patten's early paper on the embryology of *Patella* ('85), excellent as it is in many respects, leaves this part of the development nearly untouched, and the same is true of the still earlier paper of Lacaze-Duthiers ('57) and that of Kowalewsky ('83) on *Dentalium*. Everyone familiar with work of this type will appreciate the fact that to work out the cell-lineage fully would require prolonged study, and both the forms here dealt with present peculiar difficulties in the later stages. The time at my disposal has only allowed me to determine the main outlines of the cell-lineage, including details essential to the interpretation of the more important experimental results. Fortunately, however, Robert ('02) has recently published a detailed study of the cell-lineage of *Trochus*, which agrees so closely with that of *Patella* that it may be taken as a standard



of comparison. To facilitate the comparison I shall employ Robert's nomenclature, which combines certain advantageous modifications, suggested by Conklin, Mead and Child, of the system I used in 1892 in describing the cell-lineage of *Nereis*. The primary quadrants are designated as A, B, C and D (D being the posterior one), the corresponding micromeres as a, b, c and d; the coefficient (1, 2, 3 or 4) designates the number of the quartet, or in case of the basals (macromeres) the number of divisions they have undergone; each exponent denotes a subsequent division, 1 designating the cell nearer the animal pole, 2 the sister-cell nearer the lower pole. Thus, starting with the 4-cell stage, D divides into 1D and 1d; 1D into 2D below and 2d above; 1d into 1d<sup>1</sup> above and 1d<sup>2</sup> below (the primary trochoblast); 1d<sup>1</sup> into 1d<sup>1.1</sup> above (primary rosette-cell at the upper pole) and 1d<sup>1.2</sup> below (primary cross-cell); 2D into 3D and 3d; 2d into 2d<sup>1</sup> and 2d<sup>2</sup>, etc. Since in *Patella* the quadrants cannot be distinguished by simple inspection before the 32-cell stage I shall in general, where the quadrant is unknown, omit the letter. Thus the primary trochoblast is 1<sup>2</sup>, the primary rosette-cell 1<sup>1.1</sup>, a primary quartet-cell 1, 2, 3 or 4, and so on.

Both *Patella* and *Dentalium* are typical examples of the spiral type of cleavage, the former being of the symmetrical type (like *Crepidula*, *Trochus*, *Hydroides* or *Polygordius*) in which the four quadrants are of nearly or quite equal size, the latter of the asymmetrical type (like *Nassa*, *Ilyanassa*, *Unio*, *Nereis* or *Amphitrite*) in which the first division is unequal and the posterior quadrant is larger than the others until after both have been formed. *Dentalium*, further, is characterized by the formation during the first three cleavages of a large polar lobe which afterwards fuses with the posterior cell, CD, the egg passing at the first cleavage through the characteristic "trefoil stage" that so commonly occurs among mollusks (*Nassa*, *Ostrea*, etc.) and occasionally in annelids (*Myzostoma*, *Sabellaria*, *Chaetopterus*). In a preceding paper ('04) I have sketched the early cleavage of *Dentalium* and will here describe primarily that of *Patella*.

The egg of *Patella* first divides into equal quadrants, without the formation of a polar lobe; and the 4-cell stage is remarkable



*Normal Development of Patella.*

(From Acetic-Glycerine Preparations;  $\times 200$ ).

1, 4-cell stage, from upper pole; 2, 8-cell stage, from upper pole, preparing for fourth cleavage; 3, 16-cell stage, from the side (primary trochoblasts shaded); 4, 32-cell stage, from the side; 5, 48-cell-stage (transitional to 56-cell stage); 6, 48-cell stage (transitional to 52-cell), from upper pole; 7, 58-cell-stage, from upper pole, after division of the rosette-cells and establishment of the primary cross; 8, etenophore-stage, about 10 hours, from upper pole, primary trochoblasts ciliated; 9, 52-cell stage, from lower pole; 10, embryo of about 11 hours, from the right side, showing three secondary trochoblasts in the lateral gap; 11, the same embryo from the left side; 12, portion of the same, anterior view; at the opposite end are two secondary trochoblasts (primary trochoblasts stippled, secondary unshaded).



from the fact that in the early stages it often shows no cross-furrow (thus differing from *Trochus*), or if one is present it is very short (Fig. 1), though in the 32-cell stage a characteristic cross-furrow is present at the lower pole (Fig. 13). It is therefore impossible to identify the quadrants in the earlier stages without having observed the divisions from the beginning. As usual, three quartets of ectomeres are successively formed by alternating dextrotropic and leiotropic spiral or oblique divisions. The micromeres of the first quartet, often only slightly displaced towards the left, are considerably smaller than the basal cells, but are relatively larger than in *Trochus* (Fig. 2). The fourth cleavage is closely similar to that of *Trochus*, each of the upper cells dividing slightly unequally and each of the basals somewhat more unequally to form the second quartet. In the 16-cell stage (Fig. 3) the egg consists as usual of four large basal cells (2A, 2B, 2C, 2D), four smaller upper cells ( $1a^1$ — $1d^1$ ) and eight alternating cells surrounding the equatorial region. Four of these, of equal size, form the second quartet (2a—2d). The alternating four, which are somewhat smaller ( $1a^2$ — $1d^2$ ), are the *primary* trochoblasts,<sup>1</sup> by two successive equal divisions of which arise the sixteen cells of the primary prototroch. The 16-cell stage is thus closely similar to that of *Trochus*, except that the basal cells are relatively smaller while all the others are relatively larger.

The fifth cleavages are dextrotropic and symmetrical throughout the embryo, and again agree in the main with those of *Trochus*. Each of the basals divides unequally to form a cell of the third quartet, relatively somewhat larger than in *Trochus*, while each cell of the second quartet divides nearly equally (in *Trochus* this division is distinctly unequal). The primary trochoblasts divide equally, the upper cells unequally, so as to form at the upper pole a rosette of smaller cells (Fig. 6) almost identical with those in *Trochus*, but slightly larger.

The 32-cell stage thus attained (Fig. 4) is at first perfectly radially (spirally) symmetrical. From the four large symmet-

<sup>1</sup>These cells, and their products, are stippled in all of the figures.

rically placed basal cells arises the ento-mesoblast, while as usual the 28 remaining cells constitute the ectoblast.

The sixth cleavages (32-64 cells) are in the main oblique and leiotropic; but unlike *Trochus* the posterior micromeres of the third quartet depart more or less widely from the type. Proceeding from the upper pole downwards the divisions are as follows. The rosette-cells ( $1^{1.1}$ ) divide nearly equally in regular spiral order exactly as in *Trochus*, so as to form a symmetrical group of eight small cells at the upper pole (Fig. 7) which form, certainly in part and probably as a whole, the basis of the apical organ. Nearly at the same time the  $1^{1.2}$  cells divide nearly equally, so as to form the primary "cross," which, as in *Trochus*, has at this period spirally curved arms (Figs. 6, 7). The trochoblast-pairs ( $1^{2.1}$  and  $1^{2.2}$ ) divide equally, somewhat earlier than the foregoing, so as to produce four symmetrically placed groups of four equal cells (Figs. 5-7). This division takes place much earlier than in *Trochus*, and no further division occurs in the products, which become ciliated from the eighth to the tenth hour and form the primary prototroch. The second quartet cells divide at about the same time in a very characteristic fashion that is almost identical with that occurring in the nemertine egg and nearly similar to that of *Trochus*. The upper left cell ( $2^1$ ) divides slightly unequally, the smaller cell lying above and between the two adjoining trochoblast groups (Fig. 5). The lower right cell ( $2^2$ ) divides still more unequally, the smaller lower cell ( $2^{2.2}$ ) lying below against the corresponding macromere, and between the two adjoining cells of the third quartet. In *Trochus* this cell is smaller still. The egg thus attains a 56-cell stage, at which a slight pause occurs, and in the meantime a marked change occurs in one of the macromeres which, I think, is undoubtedly the posterior one, 3D. This cell rapidly passes into the interior, its outer end becoming greatly reduced, and being connected with a narrow neck with a swollen interior portion, the nucleus however still lying at the surface (Figs. 9, 13). The next cells to divide are those of the third quartet. The two anterior ones divide leiotropically, like the preceding micromeres. In the two posterior ones, however, the spindles assume a bilateral position, with the central poles

close against the outer end of 3D (Figs. 9, 13); and while I have not actually seen the division, it is nearly certain from the position of the spindles that the division is unequal. The study of a good many preparations of this stage leads me to believe that this is a constant relation.

The last cells to divide in the sixth cleavage are the macromeres, and of these 3D is the first. At the time of its division it is only connected with the surface by a very narrow neck, assuming the extraordinary appearance shown in Fig. 14. The result of this division is to form a large rounded cell, that lies quite in the upper hemisphere (shown in Figs. 15, 16) and a more superficial cell. From the conditions observed at a slightly later stage I believe the former to be 4D, the latter the primary somatoblast 4d or M; but I am not entirely certain of this identification. Slightly later the remaining macromeres divide somewhat unequally, the cells in the meantime undergoing considerable shiftings and extending further up into the egg, so that it is exceedingly difficult to identify them individually. The ectoblast-cap has now extended far down towards the lower pole, so that the macromeres are connected with the surface by narrow necks. The cell I believe to be 4d now divides symmetrically into two to form two large symmetrical cells lying between the entomeres and the ectoblast (Figs. 15, 16), which correspond with the mesoblast pole-cells as figured by Patten (*c. g.*, in his Figs. 27, 36). I have not positively traced these cells into the cœlomesoblast, but believe there can hardly be a doubt as to their nature.<sup>1</sup> At this period the large inner cell (identified as 4D) is still undivided (Fig. 15) the primary trochoblasts have developed cilia, and the apical tuft is present (10-12 hours).

Beyond this point I shall not for the present attempt to trace the general cleavage, but will pass on to some points in the later development. Patten has given figures of the trochophore of

<sup>1</sup>Sections of the trochophores of 24 hours clearly show two large mesoblastic pole-cells (one of which appears in Fig. 17) near the posterior end, from which two mesoblast-bands extend forward as figured by Patten, *c. g.*, in his Fig. 50.



*Patella* with which in the main my observations agree, though the arrangement of the cells of the prototroch is somewhat schematized. The embryo becomes ciliated at about eight to ten hours (depending on the temperature) the first cells to acquire cilia being the sixteen primary trochoblasts. For a brief period the prototroch consists of only those sixteen cells, still arranged in four separate groups (Fig. 8). The cilia are from the first arranged, not in vague patches or tufts, but in very definite oblique transverse rows, which bear a marked resemblance to the swimming plates of a ctenophore—indeed, it hardly seems forced to compare the embryo directly at this period to a larval ctenophore. At the same time, or a little later, the group of small cells at the apical pole, derived mainly, if not wholly, from the apical rosette, develops a tuft of flexible but non-vibratile sensory flagella, and constitutes the apical organ.

The ctenophore-stage is of short duration. In two or three hours several cells lying in the gaps between the four groups of primary trochoblasts also become ciliated and ultimately enter the prototroch as *secondary trochoblasts*. These cells are not more than half the size of the primary trochoblasts (a point of importance in connection with the experimental results), and at first bear much smaller cilia. There are at least three and probably four of these trochoblasts in each quadrant (with the possible exception of the posterior group, in which I have only certainly seen two of these cells), (Figs. 10-12), giving a total of 28 to 32 cells in the prototroch, to which possibly still others may be added. While I have not traced step by step the exact origin of these cells, their position in the embryo leaves little doubt that in each quadrant two of them are derived from the first quartet (*i. e.*, from derivatives of the  $1^{1,2}$  cells), and this is demonstrated to be the case by the experimental evidence. The position of the third cell (Fig. 10) shows almost beyond a doubt that it is derived from the second quartet, *i. e.*, from  $2^1$ , and probably from  $2^{1,1}$ . The experimental evidence again proves that at least one, and in some cases two, trochoblasts are derived from the second quartet. As may be seen in Fig. 10, a second cell lies next to the one described, the position of which indicates

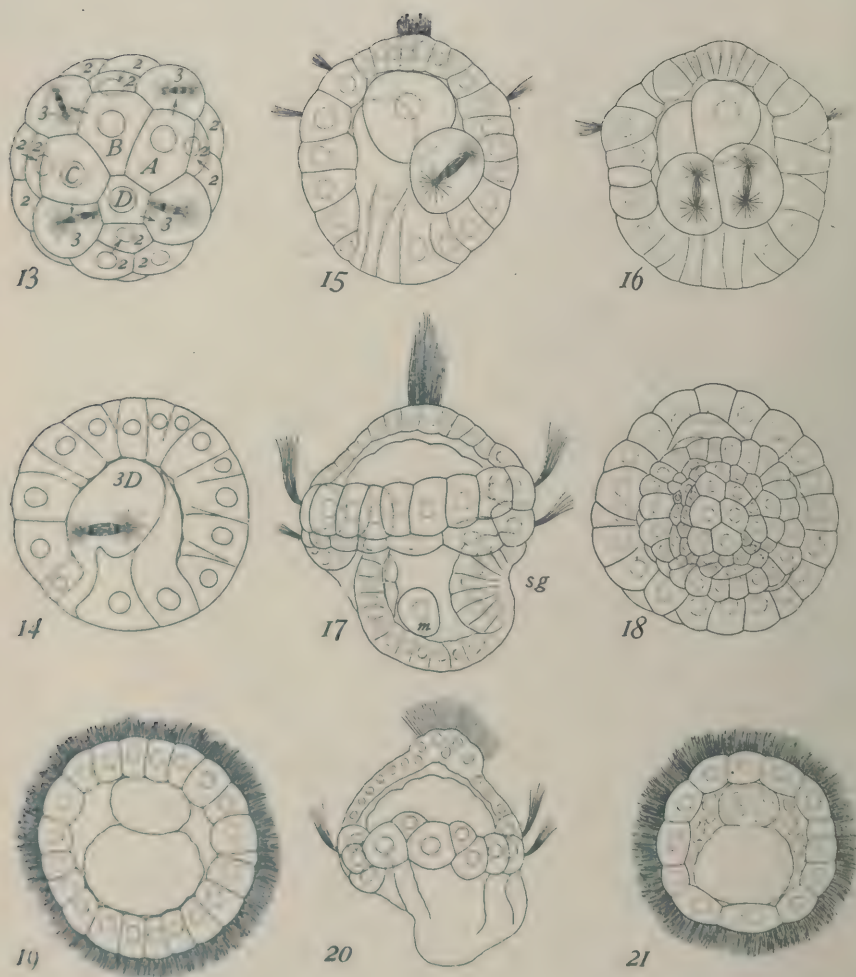


FIG. II.

*Normal Development and Larva from Egg-Fragment, Patella; x 200.*

13, 48-cell stage, lower pole; 14, larva of 9 hours, sagittal optical section, division of 3D; 15, larva of 12 hours, optical sagittal section, showing left primary mesoblast (?) in division; 16, the same larva, in frontal optical section; 17, trochophore of 30 hours, from the left side (from a total preparation, shell-gland (s. g.) and primary mesoblast (m) from a corresponding actual section), prototrochal cells in surface-view, body-wall in section; 18, larva of 20 hours, from upper pole, showing the cells as accurately as possible (some of those just anterior to prototroch could not be clearly seen and have been omitted); 19, optical section at the level of the prototroch of normal larva of 24 hours; 20, larva of 24 hours from fertilized egg-fragment that segmented like a whole egg, prototrochal cells in surface-view; 21, optical section of the same larva at the level of the prototroch; in all these figures the prototrochal cells are shown as accurately as possible.



that it may also enter the prototroch.<sup>1</sup> The 28 (32?) trochoblasts are at first arranged in two roughly alternating rows encircling the embryo slightly above the equator; and the ciliary plates of contiguous cells are still not united to form a continuous ciliary girdle (Figs. 10-12). Later, extensive shiftings of the cells occur in such wise that a principal circle of trochoblasts is formed in a single circle completely surrounding the embryo, bearing a perfectly continuous series of powerful cilia (Figs. 17-19). The cells in this row vary in number from 19 to 21—a fact of which no doubt is left by the study especially of acetic-glycerine preparations, in which the cells may be seen with schematic clearness. Posterior to this row lies a second row of smaller elongated trochoblasts, which in the dorsal region become as large as those of the principal row (Fig. 17). At this point, therefore, where in so many trochophores a gap exists in the prototroch, the ciliated belt is not only closed, but broader than at any other point. At this point the prototroch is often three cells wide; elsewhere I have not been able to distinguish three rows of cilia as figured by Patten, though three such rows are certainly present in *Dentalium*.

The trochophore of 24-30 hours (Fig. 17) is in the main similar to that of *Dentalium*, as described in my former paper, but the post-trochal region is relatively larger, the pre-trochal region less pointed, the apical tuft shorter and broader, and the apical plate less clearly marked off from the surrounding ectoblast as may be very clearly seen in sagittal section. In this respect my

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<sup>1</sup>This derivation of the prototroch in *Patella* agrees closely with that of *Ischnochiton* (Heath, '99), where two cells in each quadrant are likewise contributed from 1<sup>1,2</sup>, and two from the second quartet except in the D-quadrant, where a non-ciliated dorsal gap exists from the first. I have determined beyond doubt, I think, that at least two secondary trochoblasts are formed in the mid-dorsal line, as shown in Figs. 10-12, where there are three such trochoblasts in three of the quadrants and two in the fourth. There is further no doubt whatever that the completed prototroch is closed in the mid-dorsal line (Figs. 17-19). Robert describes the prototroch of *Trochus* as agreeing exactly with that of *Amphitrite* and *Arenicola*, no cells being derived from the first quartet except the primary trochoblasts. It appears to me, however, that his observations do not fully establish this. (Cf. the useful comparative table given by Robert at p. 420).<sub>u</sub>

larvæ seem to differ somewhat from those figured by Patten, which show an extremely distinct apical plate. The later stages are in the main similar to those described by Patten, and need not here be considered.

### III.

#### THE DEVELOPMENT OF ISOLATED BLASTOMERES.

In general, as Crampton ('96) found for the 2- and 4-cell stages of *Illyanassa*, the isolated blastomere, at whatever stage it be separated from its fellows, continues to segment essentially in the same way as if forming a part of a whole embryo; but a point on which I would lay stress is that *there is a tendency for all unequal divisions to be less unequal than in the normal development*, though this is by no means always the case, and the isolated blastomere often divides exactly as in a whole embryo. The partial character of the cleavage is also frequently masked by shifting of the cells, and the partial embryos often close, sometimes at a very early period. Such shifting or closure appears, however, to have no effect on the differentiation of the cells, as is shown with especial clearness by the history of the trochoblasts. Differentiation takes, in the main, the same course as if the cell had remained united to its fellows, and gives rise to structures that agree in a general way, and sometimes exactly with the parts to which the cells would have given rise in a complete embryo. For the sake of clearness I shall not follow the most logical order, but will present first the cases that most completely sustain the above statement—namely, the blastomeres of the first quartet. It may be premised that *all of the isolated blastomeres assume a nearly or quite spherical form before division occurs*, showing no trace of flattening on one side; and they are indistinguishable from one another except in size, and in the slightly greater transparency of the micromeres. It is also necessary to bear in mind that both in *Dentalium* and in *Patella* the eggs from different females vary very considerably in size, so that exactly corresponding blastomeres from different eggs likewise present consider-

able size variations. This accounts for certain discrepancies in the figures, which represent blastomeres from many different eggs, and possibly even from different species, though most of them are from *P. carulea*. The typical size-relation in this species, from the eggs of a single female, are shown in Fig. 100, the successive concentric outlines representing the entire egg, the  $\frac{1}{2}$ -blastomere,  $\frac{1}{4}$ -blastomere,  $\frac{1}{8}$ -macromere and  $\frac{1}{8}$ -micromere. Distinct deviations from these mean volumes will be observed in the figures.

#### A.—ANALYSIS OF THE FIRST QUARTET.

##### 1. *General development of isolated micromeres of the first quartet* ( $\frac{1}{8}$ -embryos).

As described in my preceding paper, the development of the posterior micromere of this quartet (1d) in *Dentalium* differs from that of the others in being the only one to form an apical organ. In *Patella* this is not the case, each micromere giving rise to a closed ectoblastic structure, bearing at the posterior end a group of active trochoblasts and at the anterior end an apical organ (Figs. 28-29).<sup>1</sup>

The first cleavage is slightly unequal (sometimes nearly or quite equal), (Figs. 22-24). I at first supposed the smaller cell to be the primary trochoblast (1<sup>2</sup>) since in the whole embryo this cell gives the appearance of being slightly the smaller (in *Trochus* this division is described as "nearly equal"). When, however, the entire  $\frac{1}{4}$ -blastomere segments in the calcium-free water it may clearly be seen, at least in some cases, that the larger cell is the lower one (1<sup>2</sup>), and I believe therefore the primary trochoblast is slightly larger than its fellow in the normal development. This is typically followed by an equal division of the trochoblast, and an unequal division of the other cell, giving a group that closely represents one quadrant of the first quartet in

<sup>1</sup>This has not been proved in *Patella* by isolation of all four of the micromeres from one egg (as was done in *Dentalium*); but among the numerous larvæ obtained of this type all that were closely examined possessed the apical organ.



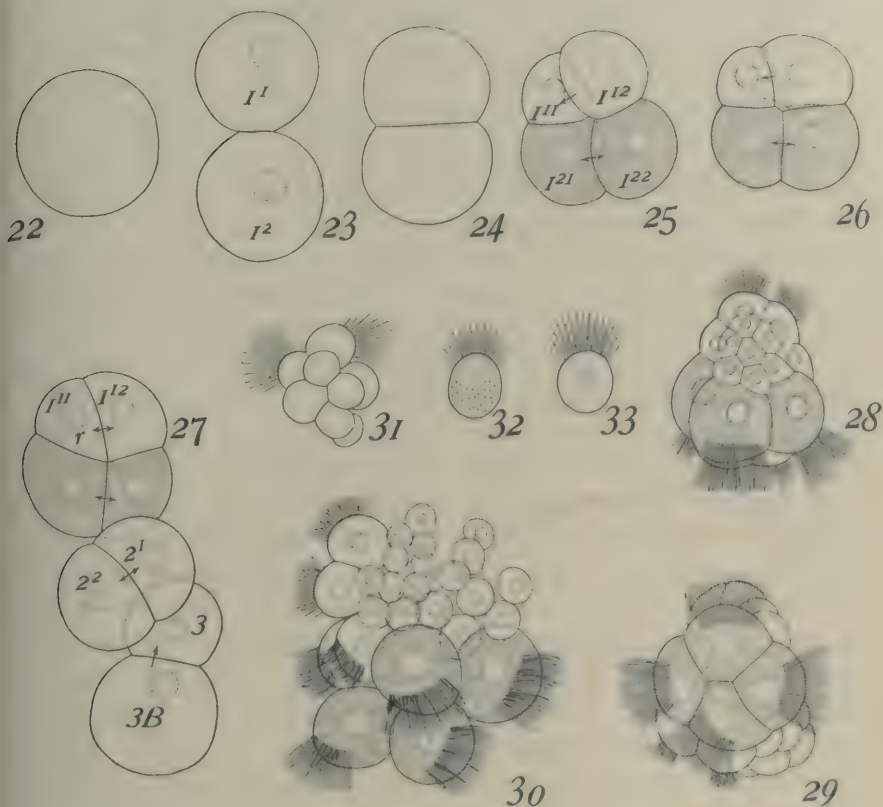


FIG. III.

*Development of Isolated  $\frac{1}{8}$  Micromeres.*

(Figs. 22-27 x 250; Figs. 28-30 x 290)

22, isolated micromere; 23, 24, first division; 25, 26, two different individuals, 32/8 stage, each with two trochoblasts, one rosette-cell, and one primary cross-cell; 27, an entire quadrant segmenting after removal from calcium-free water, products of first and second quartets somewhat separated from the basal (the first quartet group seen from the outside, the others from the inside). 28, larva of 24 hours, from  $\frac{1}{8}$ -micromere, from the side, showing trochoblasts below, apical cells above; 29, similar larva (with less regular pre-trochal region), from below, showing both primary and secondary trochoblasts; 30, loose group, from  $\frac{1}{8}$ -micromere, after 24 hours in water nearly free from calcium, primary and secondary trochoblasts, apical cells, pre-trochal ectoblast-cells; 31, group, with two apical cells, from  $\frac{1}{8}$ -micromere, after 24 hours in calcium-free water; 32-33, isolated apical cells from similar culture.

a normal 32-cell stage—*i. e.*, consists of two trochoblasts, one rosette cell ( $1^{1.1}$ ), and its larger sister cell ( $1^{1.2}$ ), from which one arises one arm of the cross (Figs. 25-27). It should be noted that the rosette-cell almost always appears somewhat too large, which is owing in part to the fact that it is less crowded than in a whole embryo, but undoubtedly in part also as to a lessened inequality in the division of  $1^1$ . Such embryos give rise to actively swimming partial larvæ, similar in a general way to the corresponding ones in *Dentalium*. These embryos do not gastrulate, but close to form pyriform ectoblastic larvæ, which bear at the larger end a group of large ciliated trochoblasts, and at the narrower end an apical organ consisting of a group of cells bearing stiffish motionless sensory hairs (Figs. 28-29). It may be clearly seen that the larger end of the embryo is formed of four primary trochoblasts, each bearing a row of powerful cilia, while just above these at one side are two somewhat smaller secondary trochoblasts. The apical organ at this period appears, in most cases at least, to include only two cells from which the sensory hairs radiate like a fan. This differs from the normal apical organ, in which the sensory hairs form a thick tuft directed straight forwards. The radiating arrangement of these hairs in the partial embryos appears to be due to the fact that the apical cells do not extend so deeply below the surface, and retain a rounded form, so that the sensory hairs spread apart like a fan, while in the normal embryo they are crowded together and assume a pyramidal shape, the free surface being considerably reduced. In the partial larvæ, too, the sensory hairs appear relatively shorter and more rigid than in the normal organ.

The composition of these larvæ is shown with great clearness by allowing the isolated  $\frac{1}{8}$ -micromere to develop in calcium-free water, the action of which is more or less restrained by the addition of a certain amount of normal sea-water. All degrees of dissociation may thus be obtained, and among the resulting cell-groups may be found forms like Fig. 30, in which the cells lie in a loose group, yet approximately retain their normal position. It is evident that each of these larvæ represents one quadrant of the products of the first quartet, including four primary trochoblasts,

two secondary ones, one-fourth of the apical organ and a group of small ectoblast cells derived from 1<sup>1,2</sup>, the whole structure closing to form a morula or blastula-like structure, but otherwise differentiating typically without gastrulating. In the aquarium these larvæ gradually disintegrate in the course of the second or third day, the trochoblasts being always the longest-lived of the cells, and often continuing to swim actively when the remainder of the larvæ has gone to pieces. I have not followed the details of the development of the corresponding isolated cells of *Dentalium*; but it is clear that their general development is closely similar. The one important difference, pointed out above, is that in *Dentalium* only the micromere from the D-quadrant develops an apical organ. As my experiments on *Dentalium* showed, the development of the apical organ in this form is determined by material that originally lies in the polar lobe, and no other conclusion seems possible than that this material is in *Dentalium* finally isolated in the posterior micromere (1d), while in *Patella* the corresponding stuff is equally distributed among the four micromeres. This is doubtless due to a different relation, in the two cases, of the original segregation pattern to the first two cleavage planes, and is perhaps connected with the absence of a polar lobe in *Patella*.

## 2. *The primary trochoblasts* (1-16, 1-32, 1-64-embryos).

Exceedingly clear and interesting results are given by the isolation of the primary trochoblasts (1<sup>2</sup>) or their products. If a single trochoblast be isolated at the 16-cell stage it divides equally twice in succession, but no further division takes place (Figs. 34-39). From the eighth to the tenth hour each of the four cells becomes ciliated and the group begins to swim. After twenty-four hours the group is swimming with great activity, and each cell is found to bear a series of powerful cilia arranged in a transverse row, exactly as in a normal embryo (Figs. 40-42). The cells vary in arrangement, sometimes lying in a single plane, sometimes having shifted so as to interlock in a rounded mass. Exactly as in a normal embryo the action of the cilia is more or



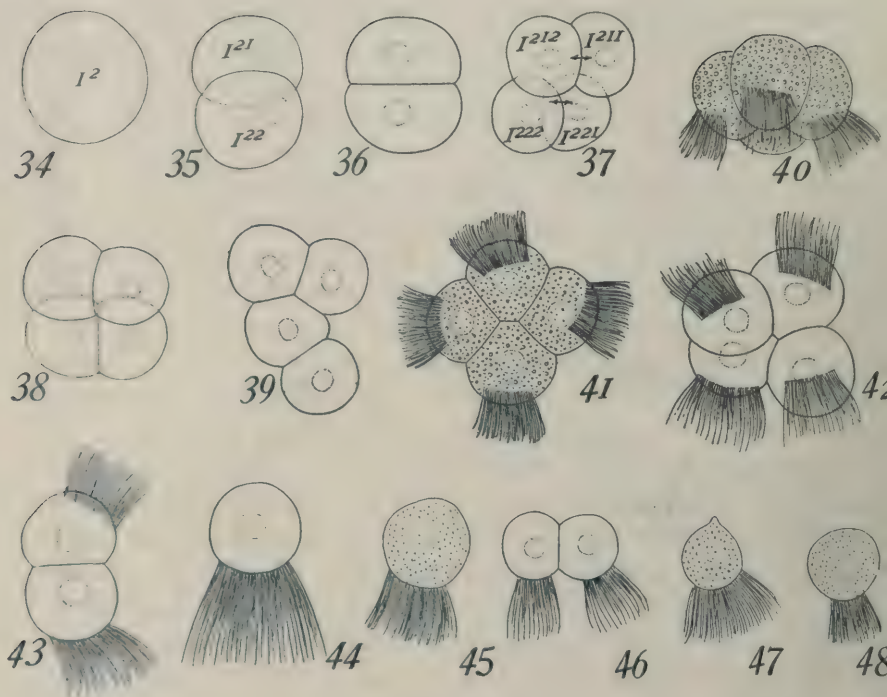


FIG. IV.

*Isolated Primary and Secondary Trochoblasts.*(Figs. 34-39  $\times 250$ ; Figs. 40-48  $\times 290$ ).

34, primary trochoblast ( $1/16$ ,  $I^2$ ), obtained by successive isolation; 35, 36, result of first division; 37-39, various forms after second division; 40, product of Figs. 34, 36, 37, after 24 hours; 41, 42, similar individuals of the same age and history; 43, pair of primary trochoblasts, 24 hours, the products of  $I^{2.1}$  or  $I^{2.2}$  ( $1/32$ ); 44, 45, single primary trochoblasts, 24 hours, products of  $I^{2.1.1}$ ,  $I^{2.1.2}$ , etc. ( $1/64$ ); 46, pair of secondary trochoblasts, 24 hours, the products of  $I^{1.2}$ ; 47, 48, single secondary trochoblasts, 24 hours.

less intermittent, sometimes ceasing wholly and again suddenly being resumed. Sudden mechanical shock frequently causes a sudden suspension of activity, followed immediately by a more vigorous activity than before. Careful study of these embryos, especially when they are dying, shows that the cilia in each cell beat in the same direction; but owing to the fact that the rows

of cilia rarely coincide in direction the group does not, as a rule, rotate in a constant direction, but irregularly.

If now the two products ( $1^{2.1}$  and  $1^{2.2}$ ) of the first division of the primary trochoblasts be separated, each divides once, and only once, thus giving a pair of cells that become ciliated and swim together like the above-described group of four (Fig. 43). If, finally, these two cells be separated at the time of ciliation—i. e., at a period corresponding with the 64-cell stage, no further division occurs, but in due time each trochoblast develops its row of cilia (Figs. 44-45) and swims singly with the fullest vigor and activity. Such single trochoblasts often rotate steadily in a nearly constant direction, proving that the action of the cilia is normally coördinated. They may live for two days or more when the action gradually ceases and disintegration occurs.

*The history of these cells gives indubitable evidence that they possess within themselves all the factors that determine the form and rhythm of cleavage, and the characteristic and complex differentiation that they undergo, wholly independently of their relation to the remainder of the embryo.* Roux's "self-differentiation" here appears in the clearest and most unmistakable form.

Similar results were obtained in *Dentalium*, but I did not in this case attempt to isolate the trochoblasts individually, but merely allowed entire eggs, or isolated  $\frac{1}{2}$  or  $\frac{1}{4}$ -blastomeres to continue their development in the calcium-free water. As in *Patella*, the result at the end of 24 hours is a chaos of more or less completely separated cells of different forms and sizes, among which are trochoblasts, actively swimming, singly or in groups. These trochoblasts fall roughly into three groups, large (Figs. 49-51), medium (Figs. 52-53) and small (Figs. 54-55). The large trochoblasts, which are considerably larger than in *Patella*, are probably primary ones, the medium and small forms secondary ones; and the difference in size among the latter suggest that as in *Patella* they may arise from different quartets. It is worthy of note that the cilia in all the trochoblasts are considerably longer than in *Patella*, and are also relatively less numerous and crowded. The small trochoblasts sometimes have as few as six cilia (Fig. 55). Sections of the entire larvæ show that the cilia

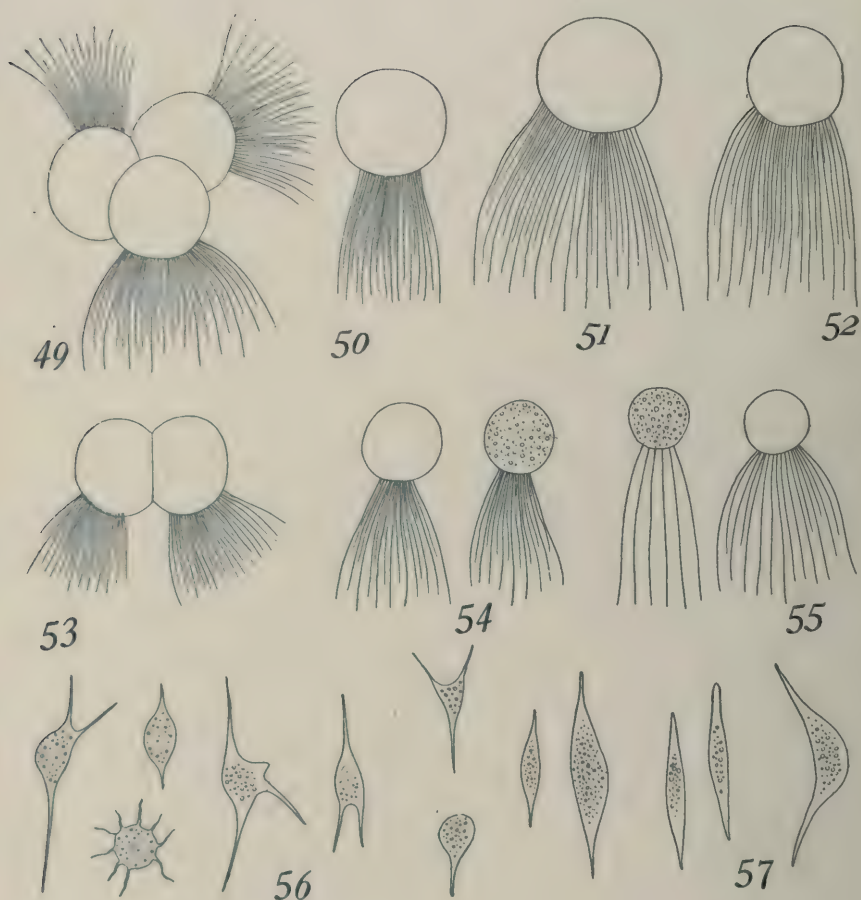


FIG. V.

*Isolated Trochoblasts of Dentalium and Mesoblast-like Cells of Patella 24 Hours:*  
x 290.

(All these obtained by leaving entire embryos in the calcium-free water for 24 hours).

49-51. large (primary) trochoblasts; 52, 53. medium (secondary?) trochoblasts; 54, 55. small (secondary?) trochoblasts; 56, group of mesenchyme-like cells; 57, group of muscle-like cells.



are grouped in small tufts (an arrangement I failed to note in the isolated cells) at the base of each of which is a very distinct deeply staining basal body; and I believe this would be an excellent object for the cytological study of the possible relation of these bodies to the centrosome.

### 3. *Development of the sister-cell ( $1^1$ ) of the primary trochoblast ( $1/16$ -embryos).*

The development of this cell, which, except for its slightly smaller size, is indistinguishable in appearance from the primary trochoblast, differs totally from the foregoing in the form and rhythm of cleavage, and in the course of its differentiation. After isolation this cell typically divides unequally to form a single rosette cell ( $1^{1.1}$ ) (though here, too, the inequality is often less marked than in the normal embryo, and sometimes disappears), and its larger sister ( $1^{1.2}$ ), (Figs. 58-60); and this is typically followed by a nearly equal division of both these cells to form a group of four, two of which obviously represent daughter-rosette cells (Figs. 61, 62). The divisions do not cease here, however, but continue, and at the end of 24 hours a larva is produced that consists of many cells and is somewhat similar to those arising from an entire micromere of the first quartet; this larva is, however, only about half as large, *and lacks the four large trochoblasts at the posterior end* (Figs. 63-64). At this end of the embryo are two trochoblasts, much smaller than those of the primary group, which obviously represent the secondary trochoblasts of the first quartet. At the narrower anterior end is an apical organ precisely like that of the micromere  $\frac{1}{8}$ -embryo, while the middle region consists of small ectoblast cells usually larger on one side than on the other. These embryos swim actively, but less vigorously than the  $\frac{1}{8}$ -forms, and, like the latter, perish in the course of the second or third day. It is obvious that the development of the  $1^1$  cell is, except for its closure, essentially the same when isolated as when it forms part of a whole embryo; and its remarkable contrast with its sister-cell, the primary trochoblast, shows most convincingly that despite their

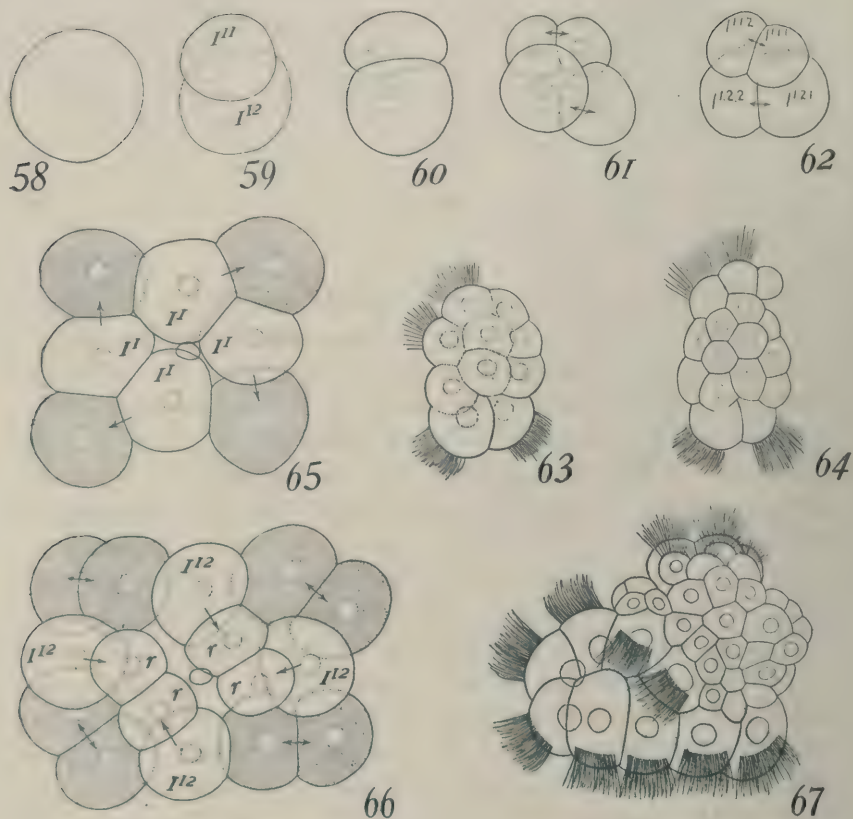


FIG. VI.

*Isolated  $I^1$  Cells and Isolated First Quartet, Patella.*

(Figs. 58-62, 65, 66 x 250; Figs. 63, 64 x 290).

58, isolated  $I^1$ ; 59, 60, two examples of first division (rosette-cell slightly too large in both); 61, 62, two examples of second division; 63, product, 12 hours, apical cells and secondary trochoblasts (somewhat smaller cells on reverse side); 64, similar larva of 22 hours; 65, first division of isolated first quartet; 66, second division; 67, product, 24 hours. (Some of the cells have been lost).

closely similar external appearance, each is from its first formation definitely specified, irrespective of its connection with its fellows.

4. *Development of isolated apical cells, and secondary trochoblasts.*

In spite of many attempts, I did not succeed in rearing singly one of the rosette cells; but isolated products of these cells, as well as isolated secondary trochoblasts, were obtained in another way. This was by allowing isolated micromeres of the first quartet to continue their development in the calcium-free water, some of the individuals being left undisturbed, others shaken to pieces from time to time by a stream from a fine pipette. In those left undisturbed for 24 hours all degrees of disintegration were observed, loose masses being found from which the trochoblasts often had broken away and were swimming about singly. In many such loose masses the characteristic apical cells could often be observed, loosely attached to their fellows at the end opposite the trochoblasts (as in Fig. 30). Those that had been shaken to pieces showed a collection of more or less completely separated rounded cells, among which spherical trochoblasts of two sizes (the larger evidently being the primary, the smaller (Figs. 46-48), the secondary ones) were actively swimming singly or in groups. Among these cells occur forms that are evidently single apical cells, since they agree exactly in size and structure with those attached to the loose masses referred to above. These cells (Figs. 31-33) are ovoidal in form, and bear on one side the characteristic non-vibratile radiating sensory processes or hairs. There is no possibility of mistaking these cells for either kind of trochoblast, since they are considerably smaller, and the appearance and arrangement of the sensory hairs (apart from their immobility) is entirely different from that of the cilia. There can, therefore, be no doubt that both the secondary trochoblasts and *typical sensory cells of the apical plate may undergo their characteristic differentiation when entirely isolated from their fellows.* Mingled with the foregoing cells are rounded, non-ciliated cells



of various sizes that are obviously isolated cells of the general præ-trochal ectoblast.

The relatively large size of the apical cells, whether completely isolated or forming part of the  $\frac{1}{8}$  or  $\frac{1}{16}$  larvæ, is a matter I have not yet fully cleared up. The apical pole of the normal larva of 24 hours (Fig. 18), seen in surface view, gives somewhat varying appearances, but clearly shows a central group of four to six larger cells. This does not exactly agree with what appears in the partial larvæ, which, as a rule, show two large apical cells, apparently of nearly equal size; it is, however, difficult to determine the exact size of the cells in the normal larva, owing to their crowding together. It seems probable that this apparent discrepancy may be due to the fact that, as stated above, the primary rosette cell is so frequently too large in the  $\frac{1}{8}$  and  $\frac{1}{16}$  embryos, and that this results in a slightly abnormal later development of its products. It is clear, however, that this does not affect in any essential way the differentiation of the apical cells.

##### 5. *Development of the isolated entire first quartet.*

In the sea-urchin Driesch (1902) has shown that both the upper and the lower quartets of the 8-cell stage may produce complete dwarf larvæ, though the two quartets show certain characteristic differences in development, proving that they are not identical. In *Patella* it is difficult to perform this operation, and still more difficult to rear the larvæ, since the cells always separate more or less after replacement in normal water. I nevertheless succeeded in obtaining a few cases. The cleavage of the isolated first quartet is essentially the same as in a whole embryo. The first division, leiotropic in all the cells, produces four upper cells ( $1^1$ ) and the four trochoblasts ( $1^2$ ) alternating with them, the eight cells forming a nearly flat plate (Fig. 65). The second division, dextiotropic in all the cells, produces a plate of 16 cells (Fig. 66) in the centre of which is the rosette, around which lie the four  $1^{1.2}$  cells and, at the margin, four groups each of two trochoblasts ( $1^{2.1}$ ,  $1^{2.2}$ ). As shown in the figures, the form and

grouping of these cells is essentially the same as in the upper half of a normal 32-cell stage; though, owing to the flattening out of the group, the normal position of the cells is somewhat modified and (as in the case figured) the cells are often rather loosely connected.

In later stages such groups invariably broke up more or less, and no larvæ were obtained which had not lost some of the cells. Nevertheless these larvæ close up more or less completely, forming irregularly pyriform structures with an apical organ at the smaller end and a group of trochoblasts at the larger one. The largest of these larvæ obtained probably represents at least three-fourths of the first quartet. This individual (24 hours) is shown in Fig. 67, drawn from a preparation; in life it swam very actively about. This larva is clearly a purely ectoblastic structure, and shows no trace of archenteron. It is of an irregular flattened pyramidal form, with an irregular group of apical cells at the narrow end, forming an unmistakable apical organ. The larger end is occupied by a group of trochoblasts, which form a somewhat irregular series around the margin, but also extend somewhat over the base. The remainder of the embryo is formed of small ectoblast cells that have on the lower side extended more or less into the basal region. The exact number of trochoblasts cannot be determined, but there are at least 14, and probably a larger number. It is clear that this larva represents a partly closed and distorted præ-trochal region, with that part of the prototroch derived from the first quartet, minus a certain number of cells that have separated. The other larvæ were similar in type, but evidently represent a smaller portion of the same region. This case, taken in connection with the other facts determined, renders it practically certain that the first quartet as a whole is here incapable of producing a complete dwarf, but gives rise to essentially the same ectoblastic structures as in a whole embryo. This result is entirely in agreement with that afterwards obtained on *Cerebratulus* by Zeleny ('04), who, at my suggestion, undertook a comparison in this form of the upper and lower quartets of the 8-cell stage—a question particularly interesting in this case since the upper quartet is larger than the lower.

The constant result of this experiment was, that while *both quartets produce closed blastulas, only the lower one gastrulates, while only the upper one produces an apical organ.*

#### 6. *Summary on the first quartet.*

*The foregoing observations are sufficient, I believe, to establish definitely the mosaic character of cleavage and differentiation in the first quartet.* This is strictly proved for the primary trochoblasts ( $1^2$ ), for their first ( $1^{2,1}$  and  $1^{2,2}$ ) and second ( $1^{2,1,1}$ ,  $1^{2,1,2}$ , etc.) products, and for the  $1^1$  cells; it is less strictly but hardly less convincingly established for the apical cells and the secondary trochoblasts. Excepting the secondary trochoblasts, the other products of  $1^1$  do not show sufficiently definite characters to allow of a similar definite proof, but it can hardly be doubted that the same conclusion applies to them, and also to the first quartet taken as a whole.

#### B.—EXPERIMENTS ON CELLS OF THE LOWER HEMISPHERE.

Isolated blastomeres of the lower hemisphere are in general much less tenacious of life than those of the upper quartet (a fact parallel to that observed by Driesch in sea-urchins), and my observations are here much less detailed. In a more general way, however, the results are entirely in agreement with the conclusions reached in case of the upper quartet.

#### 1. *Development of the isolated 1/8-macromere.*

These cells divide, at least as far as the 64/8-cell stage, as if forming part of a complete embryo. At the first division a cell of the second quartet is formed (Fig. 69-70), which at the ensuing cleavage divides nearly equally (into  $2^1$  and  $2^2$ ), while a cell of the third quartet is produced from the basal in the proper position (Fig. 71). At the next cleavage  $2^1$  divides nearly equally,  $2^2$  very unequally, forming below the characteristic small cell  $2^{2,2}$ , that lies against 3. The latter cell then divides equally or unequally (the quadrant probably being in the former case one



of the anterior, in the latter case one of the posterior ones). A group is thus produced (Figs. 72-73) which, excepting the usually lessened inequality of  $2^{1.1}$  and  $2^{1.2}$ , is practically identical with one quadrant of the lower hemisphere in the 64-cell stage (Cf. Figs. 4-5). This is followed by a division, sometimes distinctly unequal, sometimes nearly equal, of the basal to form a cell of the fourth quartet (Fig. 73).

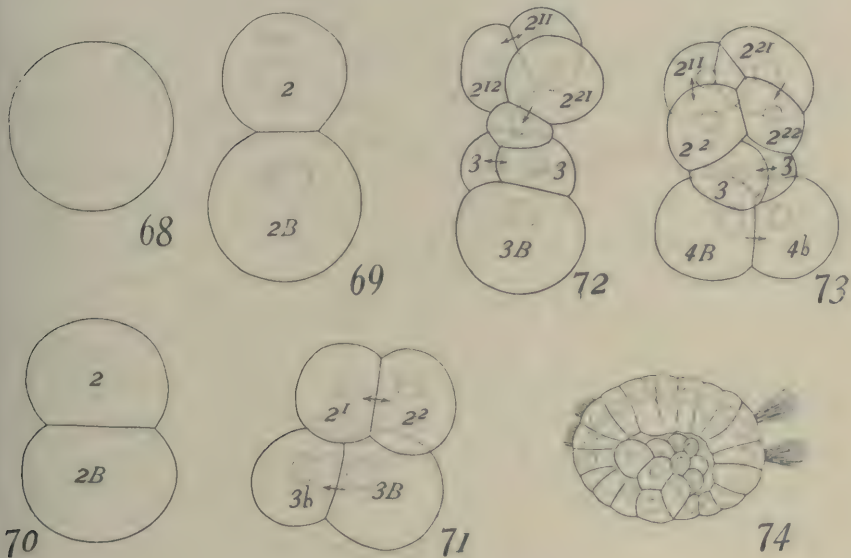


FIG. VII.

*Isolated 1/8 Basal, Patella; x 250.*

68, isolated basal (a rather small example; cf. Fig. 100); 69, 70, examples of first division; 71, 32/4-cell stage, typical; 72, 56/8-cell stage, typical 2-group, equal division of 3; 73, 64/8-cell stage, after formation of 4 (in this example the 2-group has rotated into an abnormal position); 74, product, 24 hours, showing two secondary trochoblasts (products of  $2^{1.1}$ ) and two feebly ciliated cells (pre-anal cells?).

It is exceedingly difficult to rear these embryos, many of them dying, while most of those that live break up into smaller masses. A few larvæ were nevertheless obtained, the best of which is shown in Fig. 74. Like the others, this larva has evidently gastrulated (though the entoblast-mass is relatively small, perhaps owing to the loss of some of the cells). Its most interesting features are the presence, at one end, of two cells bearing powerful and active cilia by the activity of which the larva rotates irregularly, while near the opposite end are two cells bearing much smaller and feebler ones. It is evident that the two anterior cells are secondary trochoblasts, undoubtedly those derived from the second quartet; and the fact that there are two of these may be taken as evidence that two trochoblasts are contributed to the prototroch, at least in some of the quadrants, by the  $2^{1.1}$  cells, as is indicated by a study of the normal embryos. The two weakly ciliated cells were to me at first a puzzle, since I failed to observe anything corresponding to them in the normal embryos. But it may be recalled that Patten describes and figures a ventral region, covered with fine short cilia, just anterior to the pre-anal sense-organ ('85, Figs. 47, 48, 57, etc.), and while I became aware of this when it was too late to re-examine the normal larvæ, it seems very probable that it is these cells that appear in the posterior ciliated tract of the  $\frac{1}{8}$ -macromere larva. This is sustained by the development of the  $\frac{1}{16}$  basal cells about to be described.

## 2. *Development of the $\frac{1}{16}$ -macromere.*

The  $\frac{1}{16}$  basal cell, obtained by successive isolations, divides unequally to form the third quartet cell (Fig. 75), which afterwards divides into two, while still later the fourth quartet-cell is produced from the basal (Fig. 76). Only two or three such cases were obtained, one of which developed into the larva shown in Fig. 77. While this larva could not be very clearly analyzed, it evidently consisted of an internal mass of cells (entomesoblast or entoblast) surrounded by a superficial layer of ectoblast-cells. At one end the ectoblast-cells were larger and at least one of

these bore a tuft of short, weak cilia, by means of which the larva very slowly rotated. In view of the structure of the  $\frac{1}{8}$ -macromere larva, it is probable that this ciliated cell (or cells) represents at least a part of the posterior group of cilia which I have assumed to contribute to the ventral ciliated tract in the normal larva.

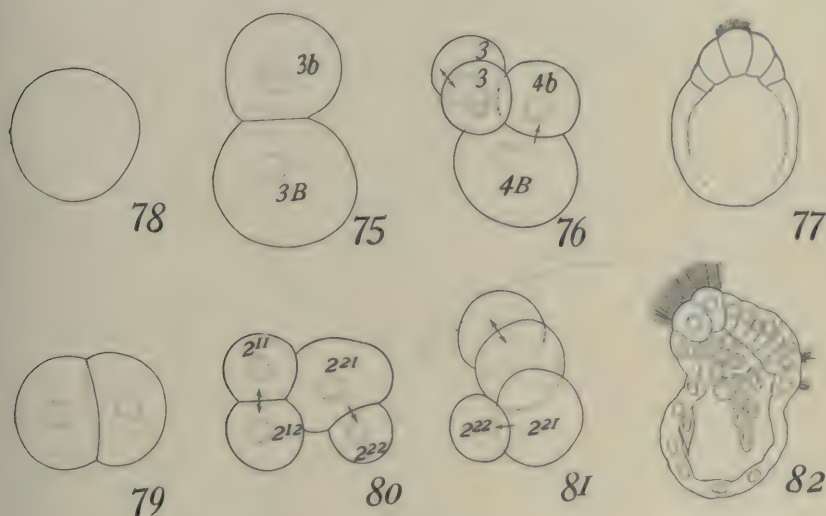


FIG. VIII.

(Figs. 75-81  $\times 250$ ; Fig. 82  $\times 300$ ).

*Isolated  $1/16$  Basal, and Isolated Cell of Second Quartet, Patella.*

75, first division of  $1/16$  basal; 76, second division ( $64/16$ ), 4 formed; 77, product, 24 hours; 78-81, cleavage of isolated 2; 82, product, 24 hours, with two trochoblasts and pre-anal (?) cells.

3. *Isolated blastomeres of the second quartet ( $1/16$ -embryo).*

These blastomeres, obtained by successive isolations, divide like the foregoing as if still forming part of a complete embryo. The first division is nearly or quite equal. In the second division one of the cells divides nearly equally, the other very unequally. Thus arises a  $64/16$ -stage (Figs. 78-81) that is closely similar to the



corresponding group in a whole embryo (Cf. Fig. 5), though these divisions are often (as in all the foregoing cases) less unequal than in a whole embryo, and their arrangement is frequently modified by shifting of the cells. At the end of 24 hours these groups produce closed ovoidal or irregular ectoblastic vesicles that swim rather slowly by means of a tuft of cilia at one end. In some cases these cilia seem to be borne by a single cell; in others I am sure there are two of these cells (Fig. 82). These cells are evidently secondary trochoblasts; and their presence is entirely in agreement with the facts observed in the  $\frac{1}{8}$ -macromere-larva described under (1), and with the fact that the normal larva clearly shows the derivation of at least one, and probably two, secondary trochoblasts from the second quartet.

There are two additional noteworthy points in these larvæ. One is the fact that, in addition to the one or two secondary trochoblasts, some of them, at least, show one or two other small patches of short and feeble cilia like those seen in the  $\frac{1}{8}$  or  $\frac{1}{16}$  macromere larva. If my interpretation of these cells is correct, this may be taken as evidence that the ventral ciliated tract arises from derivatives of both the second and third quartets.

A noteworthy point in these embryos is the presence, in some of them, of loose groups of rounded cells lying within the cavity (Fig. 82). These cells, considerably smaller than the entoblast-cells, are not improbably mesenchyme cells of the "larval mesenchyme" (pædomesoblast or ectomesoblast); and it may here be recalled that in *Crepidula*, according to Conklin ('97), the ectomesoblast is derived from the second quartet. Soon after the stage described the embryos died and disintegrated without further noticeable change.

4. *Observations on isolated cells obtained from larvæ that have developed continuously in calcium-free water.*

Beyond the facts recorded above, I have not traced the development of isolated blastomeres from the lower hemisphere. Several times I succeeded by successive isolation in separating single cells of the third and fourth quartets, and of the corresponding

basals; but in every case the embryos became abnormal or died without division. I therefore resorted to the method of allowing the eggs to continue their development for 24 hours in the calcium-free water, separating the cells from time to time by directing a rather strong jet of water upon them by means of a fine pipette. In this way the cells may be almost completely separated so as to produce what is in effect a progressive maceration of the larva without killing the cells. The result is most striking. At the end of 24 hours the whole embryo is disintegrated into its constituent cells, some of them lying in small groups, but in favorable cases many are completely isolated. The greater number of these cells are motionless and perfectly spherical, of many different sizes, and still appear to be living and in a healthy condition. Among these are swimming with great vigor numbers of trochoblasts, singly, in pairs, or sometimes in groups of four or three (Figs. 49-55). Measurements of these trochoblasts show that in *Patella* they are of two sizes, in *Dentalium*, as pointed out above, of three, the larger one agreeing perfectly with the primary trochoblasts obtained by individual isolation, the smaller with the secondary trochoblasts. Here and there can sometimes be seen a single apical cell, with its characteristic radiating sensory hairs.

Among the motionless rounded cells it is impossible to distinguish the different categories by their structure, since all have the same form and all are filled with yolk spheres. In view of the foregoing results, however, it can hardly be doubted that the largest ones are isolated entoblast-cells. But the most interesting cells are those which are not rounded but of a different form. Two kinds of such cells can be distinguished, both in *Patella* and in *Dentalium*, namely, spindle-shaped cells (Fig. 57), and branching mesenchyme-like cells (Fig. 56). The cells of both forms are relatively small, less heavily laden with yolk, and more transparent than the others. *In all these respects these cells are closely similar to the mesoblast-cells, as seen in total preparations or sections of the normal trochophore of the same age.*

These facts must be interpreted with considerable reserve; for it is well known that isolated cleavage-cells often become irregu-

lar or even amœboid, and I have sometimes observed even trochoblasts of very irregular form. But this is not the case with most of the isolated cells in *Patella* and *Dentalium*, and I am inclined to accept the probability that the cells in question may really be mesenchyme- and perhaps actually muscle-cells, that have differentiated in more or less complete isolation from their fellows. If this be considered an improbable conclusion, it should be recalled that a trochoblast is probably, to say the least, as highly differentiated as a mesenchyme cell; yet it has been strictly proved that such a cell may undergo its normal differentiation and continue for a time to perform its normally coördinated activities when completely isolated from the time of its formation. Further research specifically directed to this point will, I believe, give a positive result on this very interesting question.

5. *Summary on isolated cells from the lower hemisphere.*

The evidence derived from these cells is less detailed and complete than that derived from the first quartet; but as far as it goes gives the same general conclusion. The isolated  $\frac{1}{8}$ -macromere,  $\frac{1}{16}$ -macromere or second quartet-cell segments as if forming part of a whole embryo, and shows more clearly than do the first quartet cells that not only the form, but also the rhythm of cleavage is maintained (precisely as I showed in the nemertine); for in the cleavage of both the  $\frac{1}{8}$ - and the  $\frac{1}{16}$ -macromere the fourth quartet cell is the last to form. Only the embryos containing derivatives of the second quartet produce secondary trochoblasts, namely, those arising from the  $\frac{1}{8}$ -macromere or the second quartet-cell. While all the embryos close, only those gastrulate that contain the basal region (*i. e.*, the entoblast region). All of the three types examined develop one or two feebly ciliated cells that probably represent cells of the pre-anal ventral ciliated tract. Finally, there is some evidence, though only of an inferential character, that isolated mesoblast-cells may develop into mesenchyme-cells, possibly into muscle-cells. We may, therefore conclude that, speaking broadly, the development of cells of the lower hemisphere, like that of the upper, conforms to the mosaic principle.



## C.—DEVELOPMENT OF ISOLATED CELLS OF THE TWO- AND FOUR-CELL STAGE.

I have purposely left to the last an account of the development of the half or quarter embryos, since this is in *Patella* in some respects the least satisfactory part of the work. This is owing especially to the great susceptibility of the  $\frac{1}{2}$  and  $\frac{1}{4}$ -larvæ, which frequently break up into smaller fragments, go to pieces, or become quite abnormal during the cleavage process, so that very few satisfactory larvæ were obtained. In *Dentalium* the results are much better, since the blastomeres can be easily separated without the use of the calcium-free water; but even here my fixed material has proved insufficient for a satisfactory analysis of the internal phenomena. For these reasons the following observations remain somewhat fragmentary and must await a supplementary study in these or other forms.

1. *The partial cleavage in Dentalium.*

In my preceding paper I have described in a general way the development of isolated halves and quarters in *Dentalium*, and will here only add some details regarding their mode of cleavage, which are hardly more than a confirmation of Crampton's results on *Ilyanassa*. As in *Patella*, these earlier blastomeres, like the later ones, become perfectly spherical after isolation before cleavage begins; their characteristic partial cleavage must therefore be due to internal factors and not to their shape.

The AB (anterior) half, which shows only an upper white polar area (Fig. 83), segments equally into two, with no trace of a polar lobe (Figs. 84-85), and then forms by dextrotropic divisions two slightly smaller micromeres of the first quartet, which are composed entirely of white material (Fig. 86). The following division (Fig. 87) is like that occurring in half an egg, the upper cells dividing slightly unequally to form below the two primary trochoblasts ( $1a^2$  and  $1b^2$ ) and above the two upper cells ( $1a^1$  and  $1b^1$ ). The lower cells in the meantime produce the two cells of the second quartet (2a, 2b) in characteristic

fashion, these being likewise composed mainly or wholly of white material (Fig. 87). Beyond this point (16/2 stage) I have not followed the divisions.

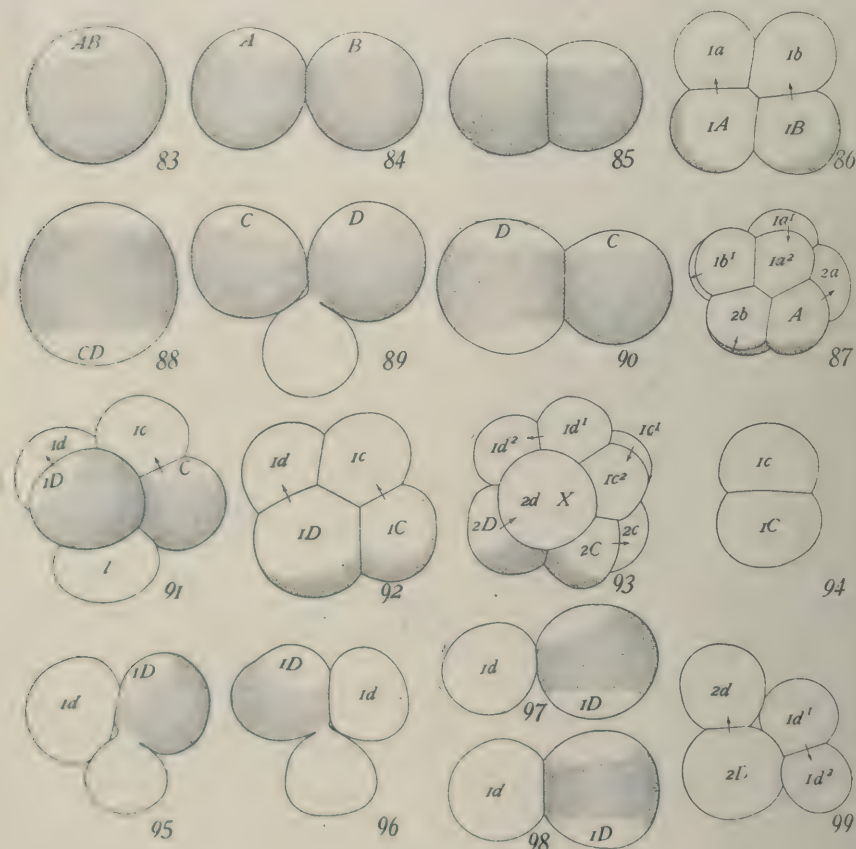


FIG. IX.

*Cleavage of Isolated Blastomeres in Dentalium.*

83, 88, isolated AB and CD halves, before division; 83-87, cleavage of AB-half; 84, 85, 4/2-cell stage, from the side; 86, 8/2-cell stage, from the side; 87, 16/2-cell stage, from the side; 88-93, cleavage of CD-half; 88-90, first cleavage (second polar lobe) and resulting 4/2-cell stage, from the side; 91, second cleavage (third polar lobe) from the side; 92, resulting 8/2-cell stage, oblique view, from the side and above; 93, 16/2-cell stage, with first somatoblast, 2d (X) obliquely from the side; 94, 2-cell stage of C-fourth; 95-99, cleavage of D-fourth; 95, 96, trefoils; 97, 98, 8/4-cell stages; 99, 16/4-cell stage (seen from the inner side, so as to appear reversed).

The CD half, which clearly shows both upper and lower white polar areas (Fig. 88), forms a polar lobe from the lower white area and passes through a trefoil stage, nearly similar to that of a whole egg (Fig. 89). Measurements show, however, that the polar lobe is always proportionately larger than in a normal trefoil, being often as large as in a whole egg, though sometimes more or less reduced. The lobe subsequently fuses with the posterior cell, D, producing a  $4\frac{1}{2}$ -cell stage closely similar to a normal 2-cell stage, except that the inequality is greater (Fig. 90). At the second cleavage the polar lobe forms again (Fig. 91) from the larger cell, D, which divides unequally and dextrotopically to form 1d, while the smaller cell, C, divides slightly unequally to form 1c. As in the whole egg the polar lobe then fuses with D, producing an  $8\frac{1}{2}$ -cell stage that is essentially like the posterior half of a normal 8-cell stage (Fig. 92). The following cleavage is especially interesting, corresponding again with the divisions in the posterior half of a whole egg (Fig. 93). All the divisions are leiotropic. The two upper cells divide slightly unequally to form the two primary trochoblasts ( $1c^2$ ,  $1d^2$ ) and the slightly larger upper cells ( $1c^1$  and  $1d^1$ ). From 1C arises the right cell (2c) of the second quartet, while from 1D arises the first somatoblast (2d), which is as large as in a whole embryo, *and in like manner is mainly formed from the lower white area in 1D*. The  $16\frac{1}{2}$ -cell stage has, therefore, exactly the same origin and composition as the posterior half of a whole egg, consisting of six white ectomeres ( $1c^1$ ,  $1d^1$ ,  $1c^2$ ,  $1d^2$ , 2c and 2d), of which 2d is the largest, and of two macromeres (2C, 2D), which contain all of the pigment and show each an upper white area (Fig. 93).

The history of isolated  $\frac{1}{4}$ -blastomeres is entirely analogous. The A, B or C quadrant typically divides slightly unequally, without a polar lobe (Fig. 94), the smaller cell being composed of white material and the pigment remaining in the larger; but cases are not infrequent in which the division is nearly or quite equal. The D-quadrant, on the other hand, forms a polar lobe, which, as in a whole embryo, is typically much smaller than either the first or the second (Figs. 95-96). The 2-cell stage is very un-



equal, the small cell (1d) being pure white, the larger showing both upper and lower polar areas (Figs. 97-98). At the second division (virtual fourth) the second somatoblast (2d) forms from the lower polar area, while the micromere produces the single trochoblast (1d<sup>2</sup>), and the corresponding larger upper cell 1d<sup>1</sup> (Fig. 99). Beyond this the cleavage was not followed in detail. It is noteworthy that in the divisions both of the halves and the fourths the normal inequality of the cells is frequently reduced, and this is frequently expressed by a reduction in the size of the polar lobe, both in the CD-half and the D-fourth—indeed, I have seen only a few D-fourths in which the polar lobe was of full normal size, and the first division of these cells is frequently irregular and abnormal. This is doubtless due in part to shock, perhaps also to the effect of the calcium-free water when this is used. Nevertheless, I think it probable that the effect may also be due in part to disturbances in the arrangement of the cytoplasmic materials, which may possibly be interpreted as a regulative phenomena.

## 2. *The partial cleavage in Patella.*

The cleavage of isolated halves or fourths in *Patella* is entirely in agreement with the foregoing in being strictly partial in character, but I wish especially to emphasize the fact that, precisely as I showed in *Cerebratulus*, two general types exist, in one of which the cells so shift as to produce a closed embryo from the beginning, while in the other the blastula is at first widely open on one side. The point is important because the effect of the displacement in the closed type is to shift the primary trochoblast-groups more or less widely, sometimes to opposite sides of the embryo, while in the open type they remain in nearly their normal position. Nature thus performs an experiment in the displacement of the blastomeres closely similar to those carried out by Fischel on the ctenophore-egg, and a corresponding result is produced that clearly shows the differentiation of the cells to be independent of their position in the embryo.

In the following description the typical case is described; attention is again called to the fact that the unequal divisions are frequently less unequal than in the normal and sometimes become quite equal.

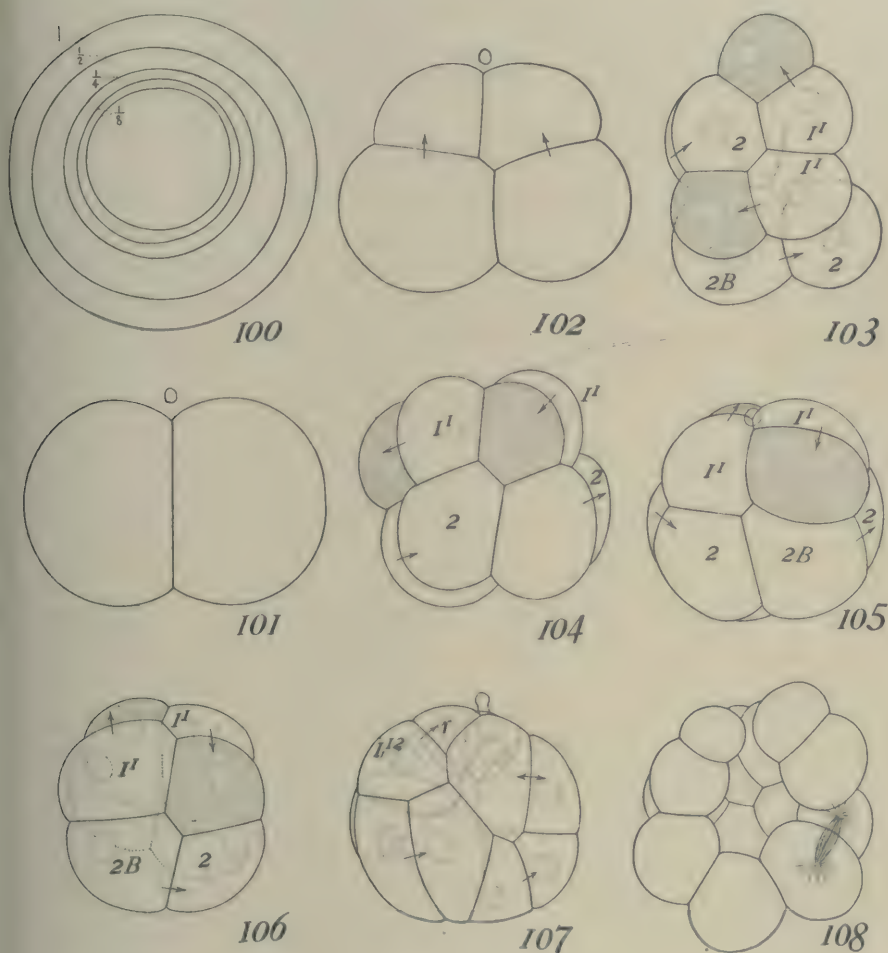


FIG. X.

*Isolated  $\frac{1}{2}$ -Blastomeres, Patella; x 250*

100, successive camera outlines, showing relative sizes of whole egg, and the  $\frac{1}{2}$ ,  $\frac{1}{4}$  and  $\frac{1}{8}$ -blastomeres; 101, 4/2-cell stage; 102, 8/2-cell stage; 103, 16/2-cell stage, nearly typical open type, from above; 104, slightly less open form from the side; 105, 106, 16/2-cell stages, closed type; 107, 32/2-cell stage, closed type; 108, open blastula, from the open side.

The isolated  $\frac{1}{2}$ -blastomere first divides equally (Fig. 101), then unequally and dextiotropically, so as to form two slightly smaller micromeres, displaced towards the left (Fig. 102). Up to this point the embryo remains strictly a half of the corresponding 8-cell stage. At the succeeding division the differences between the open and closed types become apparent. In the former case, as shown in Figs. 103-104, the divisions may occur nearly typically, though frequently the cells become more or less displaced. In the second case the cells shift during the division, so as to fit accurately together; and, as is clearly shown in Figs. 105, 106, the two trochoblasts (shaded) may thus come to lie on opposite sides of the embryo, as is also the case with the two cells of the second quartet. I have not followed out in full the later cleavage of these larvæ, which are very puzzling in both cases, owing to either the initial or subsequent shiftings. But so much is certain, that from the open type may arise an open blastula (Fig. 108), while the closed type remains closed; and the effects are clearly shown in the resulting larvæ. Fig. 107 shows a closed  $32/2$ -cell stage, with the polar body in position. This embryo is difficult to analyze in detail, but very clearly shows two rosette-cells above, with the corresponding  $1^{1,2}$  cells, and on each side are two cells that doubtless represent the daughter-trochoblasts. The eight cells of the lower hemisphere are more difficult to identify, and the cell-connections shown in the figure are only inferred.

The isolated  $\frac{1}{4}$  blastomere first divides unequally, forming a micromere above, a macromere below (Fig. 109); and this is followed by a leiotropic division identical with that occurring in a single quadrant of a whole embryo (Fig. 110). The  $16/4$ -cell stage then divides dextiotropically, producing a  $32/4$ -cell stage that may pretty accurately correspond with a single quadrant of the normal 32-cell stage. Fig. 111 shows this stage of the same individual shown in 110; this differs from a single quadrant of a whole 32-cell stage only in the fact that  $2^1$  has extended upwards somewhat, so as to separate  $1^{1,2}$  from  $1^{2,2}$ . Fig. 27 shows a  $32/4$ -cell stage that has separated somewhat (the cells are shown exactly as they lay). Every cell is of correct proportion and po-



sition, except that one of the groups of four has turned over (doubtless during the removal with the pipette), so that the upper group presents to view the outer, the lower one the inner, side; while the rosette-cell is somewhat too large. Both the cases figured represent the open type, which appears to be the rule in the quarter cleavage.

### 3. *The half and quarter larvæ in Patella.*

The detailed study of the larvæ derived from the  $\frac{1}{2}$  or  $\frac{1}{4}$ -blastomeres presents many practical difficulties. While the early cleavage of these embryos is easily determined, the later stages are exceedingly difficult to follow, owing to the shiftings of the cells, the more or less complete closure of the embryos, and the great number of defective or monstrous forms, I must admit that as far as *Patella* is concerned, and in some respects in *Dentalium* also, the following account is far from satisfactory, especially in regard to the most interesting question of all, that of the meso-blast; but since I may have no opportunity to complete it at present, I desire to record some observations which may at least open the way for a more adequate study in the future.

The most essential point has been recorded in my preceding paper, namely, that in *Dentalium* neither the  $\frac{1}{2}$ - nor the  $\frac{1}{4}$ -blastomere is able to produce a perfect dwarf larva; and, further, that the AB and the CD halves show definite and constant differences, the former lacking both the post-trochal region and the apical organ, while both these structures are present in the CD larva. In like manner, among the quarter larvæ only the D-fourth produces these two structures, which are entirely lacking in the A, B or C-fourths.

In *Patella* the corresponding comparison is far more difficult, owing partly to the equal size of the halves or quadrants, but more especially to the even greater difficulty of rearing the larvæ, which very frequently go to pieces during the late cleavage stages, and invariably become irregular and monstrous during the second day, and finally disintegrated before the larval characters become clearly marked. My observations clearly show one point,

however, in the comparison of the two halves from the same egg, in which *Patella* differs from *Dentalium*, namely, that *both halves develop an apical organ*; and while this has not been directly proved for the four quarters, the fact, described above, that any isolated micromere of the first quartet may develop an apical organ leaves practically no doubt that the same is true for the quarters. The basis of the apical organ in *Patella* must, therefore, be symmetrically divided by the first two cleavages, while it remains undivided in *Dentalium*, remaining as a whole in the D-quadrant. This is possibly correlated with the fact that the apical rosette, formed at the fifth cleavage of *Patella* and *Trochus*, fails to appear in *Dentalium*, where the  $1^{1.1}$  cells are as large as their sister-cells  $1^{1.2}$ .

In *Dentalium*, as described in my first paper, the larvæ invariably close sooner or later, and the prototroch, in most if not all cases, closes also to form a complete belt encircling the body. In *Patella*, however, this is not always the case; and frequently the  $\frac{1}{2}$ -larvæ of 24 hours show the prototroch as an area of characteristic trochoblasts extending around one side only, terminating abruptly to leave a space occupied by much smaller non-ciliated cells.<sup>1</sup> (Figs. 117, 118). In other half larvæ the prototroch appears as a complete belt, in still others as a more or less irregular or interrupted structure.

An examination of the earlier ciliated stages, combined with the results obtained with isolated trochoblasts, gives the obvious explanation of these differences. In those of the earlier larvæ (8-10 hours) that are still open on one side (and hence must have been derived from the open type of cleavage) two adjoining groups of trochoblasts are found on one side, leaving a space on the opposite side free from trochoblasts (Fig. 114). In the closed embryos, on the other hand, two corresponding trochoblast groups are formed on opposite sides of the embryo, with only rather narrow gaps between them (Figs. 113-115-116). Both these types may be represented in twins from the same egg, a case which I am fortunately able to show by Figs. 113 and 114

<sup>1</sup>In agreement with Crampton's observation that the  $\frac{1}{2}$ -larvæ of *Hyanassa* form "a partial circle of cilia" (96, p. 9).

(from acetic-glycerine preparations). Of these twins one (Fig. 113) is closed and shows the gastrulation well advanced (the superficial ectoblast-cells of the lower hemisphere are only in part shown). This larva shows very clearly the two groups of primary trochoblasts on opposite sides of the egg, at  $t$  and  $t$ , with at least two secondary trochoblasts lying between them on each side, the general arrangement being similar to that shown in Fig. 116.<sup>1</sup> The twin larva (Fig. 114) is still widely open on one side; and while the small ectoblast cells have closed in to fill the gap above, the two primary trochoblast groups lie at one side, leaving a wide gap occupied by smaller cells. Fig. 115 is a  $\frac{1}{2}$ -larva, which, though somewhat asymmetrical, is clearly of the closed type (the superficial post-trochal ectoblast-cells are shown only in optical sections at the sides); and here, too, the primary trochoblast groups lie on opposite sides of the larva.

It is hardly possible to doubt that these two types of larvæ arise from the open and closed types of cleavage, the trochoblasts having undergone their normal differentiation whether displaced or not. This has not been strictly proved by isolation experiments; but in view of the demonstrated fact that the trochoblasts differentiate typically if wholly separated from their fellows, there can be no doubt, I think, of the interpretation offered. It is quite clear that in this case *the prospective value of the cell is not a function of its position*, but is dependent on its internal organization irrespective of its position. This result is exactly analogous to those obtained by Fischel ('98) by displacing the micromeres of the ctenophore egg—an operation that, as he shows in the most convincing manner, leads to a corresponding displacement of the rows of swimming plates in the larva.

With the  $\frac{1}{4}$ -larvæ in *Patella* I had little success, since they almost invariably broke apart into smaller fragments. A very few nearly complete larvæ of 24 hours were, however, obtained, one of which is shown in Fig. 112. This larva shows a central mass of rather large rounded cells completely surrounded by ectoblast, and has evidently gastrulated. At one side is a very distinct group

<sup>1</sup>The latter larva apparently shows five primary trochoblasts on one side—a fact for which I cannot account.



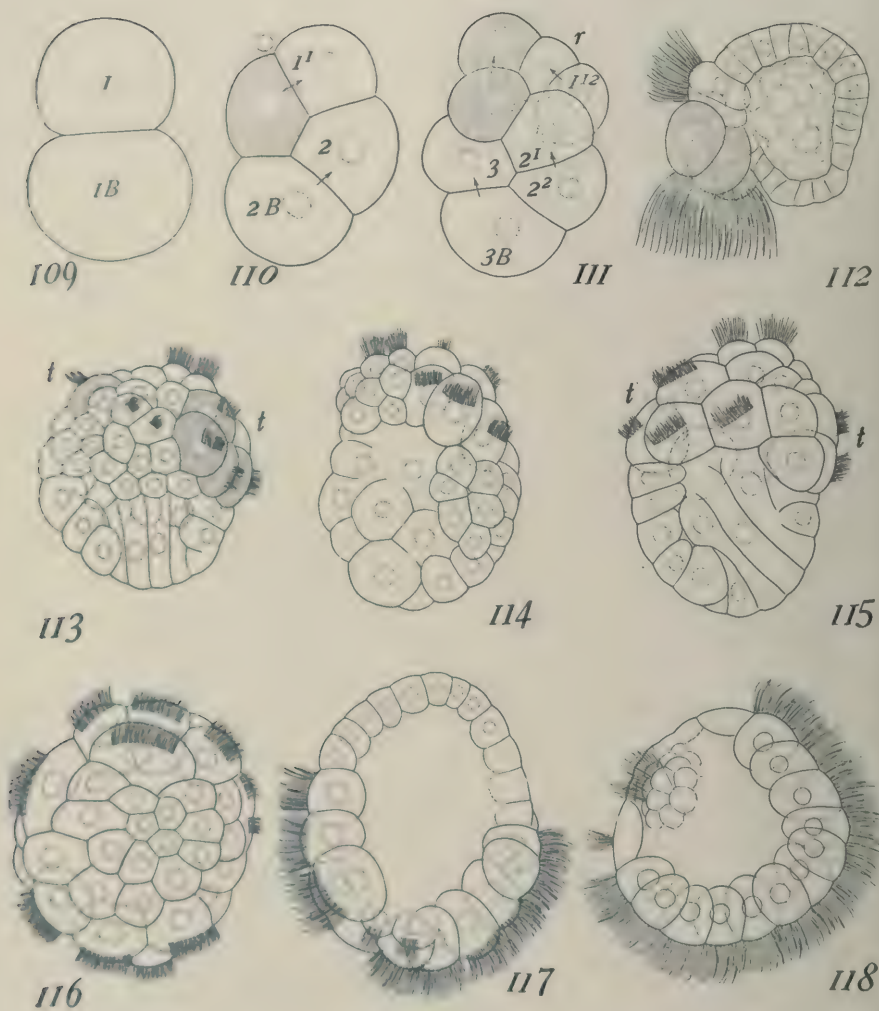


FIG. XI.

*1/4 and 1/2 Larva, Patella; x 250.*

109-111, cleavage of isolated 1/4, open type; 112, resulting larva, 24 hours; 113, 114, twin larvae, 9 hours, 113 of the closed type, 114 of the open; 115, closed 1/2-larva, 9 hours; 116, closed 1/2-larva, 11 hours, apical view; 117, 118, products of open type, 24 hours.

of six trochoblasts. Four of these, bearing powerful rows of cilia, are evidently primary trochoblasts; two, lying in front of the last, are much smaller, and are probably secondary trochoblasts from the first quartet. Those of the second quartet have either failed to develop or have broken away from their connections (as very often occurs with all the trochoblasts owing to their activity). No apical organ was seen in this larva; but I observed an apical organ in several less normally developed individuals, and since the apical organ constantly appears in the  $\frac{1}{8}$ -micromere larva, there can be no doubt that it may appear also in the  $\frac{1}{4}$ -larvæ.

Perhaps the most interesting question presented by these larvæ is whether the AB and the CD half-larvæ differ in respect to the mesoblast; for if the mosaic principle holds for this structure, one should expect to find cœlomesoblast only in the CD half. For the present I can give no certain answer to this question, further than to state that in *Dentalium* the two larvæ certainly differ to some extent in respect to the mesoblast, and there is possibly some reason to conclude that they do also in *Patella*.

In the latter form some of the larvæ show a large rounded cell in the upper region of the central mass (the dotted outline in Figs. 113 and 115) which does not appear in others; and this difference distinctly appears between the two twin larvæ shown in Figs. 113 and 114. This cell is possibly the primary mesoblast, 4d; but it may also represent the large rounded cell which I have considered to be 4D in the normal larva (Figs. 15-16). This evidence, unsatisfactory as it is, is mentioned as an indication that the internal structure of the two half-larvæ shows distinct differences in *Patella*. In *Dentalium* the evidence is somewhat better, but still far from adequate, owing to paucity of material and the confused appearance of the inner cell-mass, as seen either in total preparation or in sections. Sections of the CD larvæ nevertheless show groups of smaller and irregular cells lying between the large entoblast-cells and the ectoblast, and there is little doubt that these represent in part the cœlomesoblast. Sections of the AB larvæ are in general closely similar to those of the lobeless larvæ described in my preceding paper, but



in some cases distinctly show a few small cells lying between the gut and the ectoblast. The only conclusion that I am justified in drawing is that the mesoblast cells are more numerous in the CD larva than in the AB, and additional material will be necessary to determine the point. When, however, we consider the evidence, not entirely conclusive but still fairly definite, given in my preceding paper, that the material of the polar lobe (which passes only into the CD half) is necessary for the production of the cœlomesoblast, I think it may be concluded with some probability that the mesoblast-cells (if they be such) found in the AB half represent a portion of the larval mesoblast or ectomesoblast, and that the cœlomesoblast is represented only in the CD half. I hope in the near future to obtain additional material that may afford a more definite conclusion.

#### IV.—SUMMARY.

*(This Applies Primarily to Patella.)*

1. Isolated blastomeres of any stage segment essentially in the same manner as if still forming part of a complete embryo, with a tendency, however, for all unequal divisions to be less unequal than in the normal. The partial form of cleavage is frequently masked by shifting of the cells.

2. All of the partial embryos, if of sufficient size, tend to close to form morula- or blastula-like structures; but these only gastrulate if they contain entoblast material from the basal cells. Apart from such closure all of the cells, and their products, as far as examined, differentiate typically, regardless of their relative position or of complete isolation from their fellows.

3. Isolated  $\frac{1}{8}$ -micromeres produce pyriform larvæ, bearing at one end an apical organ, at the other a group of four primary and two secondary trochoblasts. In *Dentalium* the apical organ is produced only by the posterior micromere, 1d.

4. Isolated primary trochoblasts ( $1^2$ ) divide twice and produce four typical ciliated prototrochal cells. Isolated first products of the primary trochoblasts ( $1^{2,1}$ ,  $1^{2,2}$ ) divide once and pro-

duce a pair of typical prototrochal cells. Isolated second products ( $1^{2.1.1}$ ,  $1^{2.1.2}$ , etc.) undergo no further division, but differentiate singly into typical prototrochal cells.

5. Isolated  $1^1$  cells produce embryos bearing at the anterior end an apical organ, at the other two secondary trochoblasts.

6. Isolated products of the  $1^1$  cells differentiate into typical sensory cells of the apical organ, into secondary trochoblasts, and into less differentiated ectoblast cells.

7. Isolated  $\frac{1}{8}$ -macromeres produce closed embryos that gastrulate and bear at one end one or two secondary trochoblasts, and at some other point a small group of feebly ciliated cells, probably representing the pre-anal ciliated cells of the normal larva.

8. Isolated  $\frac{1}{16}$ -macromeres produce closed embryos that gastrulate, bear no trochoblasts, but have feebly ciliated cells, as in 7.

9. Isolated cells of the second quartet produce closed ectoblastic embryos bearing one or two secondary trochoblasts, and one or two feebly ciliated cells, that probably also represent part of the pre-anal tract. These embryos do not gastrulate, but may form mesenchyme-like cells.

10. Isolated  $\frac{1}{4}$ -blastomeres produce embryos that gastrulate, produce four primary trochoblasts, at least two secondary ones, and an apical organ.

11. Isolated  $\frac{1}{2}$ -blastomeres produce, in *Patella*, larvæ bearing an apical organ, and a prototroch, either open or closed, according to the mode of cleavage. In *Dentalium* only the CD half produces an apical organ and a post-trochal region, and probably only this half produces cœlomesoblast.

12. The development of both *Patella* and *Dentalium* is essentially a mosaic-work of self-differentiating cells.

#### V.—DISCUSSION OF RESULTS.

The experimental results brought forward in this paper and the preceding one seem to me to establish definitely the principle of mosaic development in the case of the mollusks *Dentalium* and *Pa-*

tella, and to place the study of cell-lineage on a new and firmer basis. Clearly as the exquisite adjustment between the cleavage-process and the operations of morphogenesis has been revealed by the descriptive-comparative study of cell-lineage, it appears in still stronger relief in the light of the experimental proof that the cleavage-pattern, as a whole and in detail, is the visible expression of an actual distribution of specific morphogenic factors among the cells.

Although Crampton's initial, and hitherto almost unique, experiments on this type of development had led to the expectation that some evidence of cell-specification and self-differentiation would be found, I confess that I was not prepared to find that evidence so circumstantial and consistent. The evidence in *Patella* that the cleavage-cells are definitely specified from the time of their first formation, and that they undergo self-differentiation without essential modification through their relation to the other cells, is demonstrative in the case of the cells of the first quartet, at least as far as the 16-cell stage, as shown by the development of isolated entire micromeres at the 8-cell stage, and of their products  $1^1$  and  $1^2$  at the 16-cell stage. It is no less demonstrative in the case of the products of the primary trochoblasts isolated at the 32- and 64-cell stages; and inasmuch as cells of the apical organ derived from the  $1^{1,1}$  cells, and secondary trochoblasts derived from the  $1^{1,2}$  cells, also differentiate typically when the isolated micromere is allowed to segment continuously in the calcium-free water, and the cells are separated more or less completely after every division, the conclusion is unavoidable that these cells, too, may undergo their characteristic development in complete isolation from their fellows. Less detailed, but hardly less convincing, is the evidence derived from the isolated  $\frac{1}{8}$  basal, the  $\frac{1}{16}$  basal, or the isolated second quartet-cell; and it can hardly be doubted that the individual products of these respective cells are, like those of the first quartet, definitely specified in greater or less degree.

The general conclusion thus reached in the case of *Patella* is sustained by the development of larger masses of cells derived from the earlier stages both of *Patella* and of *Dentalium*. The



entire first quartet of *Patella*, when isolated, produces a mass of ectoblast-cells, which, though it closes, does not gastrulate, but undergoes essentially the same differentiation as if it formed the upper hemisphere of a complete larva. The isolated quadrant of a 4-cell stage gastrulates, produces a group of trochoblasts and an apical organ, the latter structure appearing apparently in any of the quadrants in *Patella*, while in *Dentalium* it is restricted to the D-quadrant. In *Dentalium*, further, only the D-quadrant produces a post-trochal region, which is due to the fact that this quadrant alone contains the material of the lower polar area from which arises the somatoblasts. Finally, the two halves of the 2-cell stage gastrulate, but (at least in *Dentalium*) differ widely in their later development. Both in *Dentalium* and in *Patella* the half-embryo forms a prototroch, which in the former seems always to close to form a complete ring, but in *Patella* frequently remains open at one side, forming a half ring. In *Patella* both halves form an apical organ; in *Dentalium* only the CD-half. In *Dentalium*, finally, only the CD-half forms a post-trochal region, for the same reason as in case of the D-quadrant. It is probable, further, that only the CD-half and the D-quadrant produce cœlomesoblast. This conclusion has not thus far been satisfactorily established by direct examination of the half-embryos, but is indirectly rendered very probable through the observations on the lobeless larvæ recorded in my preceding paper.

The foregoing facts constitute a strong body of *prima facie* evidence that the entire cleavage-pattern in the molluscan egg represents (with certain reservations considered beyond) a mosaic-work of self-differentiating cells, exactly in the sense of Roux's general conception.<sup>1</sup> The proof is indeed entirely complete in

<sup>1</sup>Here, and in all that follows, I exclude from that conception the hypothesis of qualitative nuclear division. It should be borne in mind that Roux himself expressly stated as early as 1893 that this hypothesis did not form a necessary part of his conception. "*Die beiden Annahmen*" (nuclear idioplasm, distribution by qualitative division) "*sind jedoch nicht unerlässlich notwendige Glieder meiner in ihren wesentlichen Theilen experimentell erwiesenen Auffassung;*" ('93.2, p. 874, Italics in the original). This fact has been ignored by many of Roux's critics, in spite of the fact that some of his most important contributions to experimental embryology have been specifically

the case of only a few kinds of cells. It is evident that the limitations of potency vary in different cells—the  $r^1$  cells, for example, contain more complex potencies than the  $r^2$ —and it is quite possible that dependent or correlative differentiation may play a larger part in the development than my experiments have thus far shown. But the proof by experiment of definite specification and self-differentiation in only a few categories of the early cleavage-cells establishes a principle that is to be reckoned with as a most important factor in the whole problem of embryonic differentiation. If, in considering some aspects of this problem, I again take up a discussion that has been so prolonged, it is because I believe that the importance of the principle of mosaic-development, and of the nearly related one of specific formative or determining stuffs, has received insufficient recognition by many embryologists, and by some has been prematurely discredited. That a reaction is well under way will be evident to every reader of Fischel's ('03) excellent recent discussion of development and differentiation, the essential conclusions of which agree closely with those earlier stated in a brief form in my paper on cleavage and mosaic-work ('96, appended to Crampton's paper), and more fully considered in the discussion of my nemertine results ('03); the agreement with the conclusion reached in this and the preceding paper is still closer. In full harmony with the same general conception are the important cytological results of Lillie ('99, '01) and Conklin ('98, '99, '02) cited in the two preceding papers.

The long continued discussion of the mosaic-theory of development that followed its first definite formulation by Roux in 1888, in the course of which Roux so ably defended his position, has been greatly prejudiced by the fact that the experimental analysis of cleavage was at first confined to the so-called "indeterminate" types of cleavage, such as those of the echinoderm, the medusa and *Amphioxus* (it may for the time be left an open question whether that of the frog should be placed in the same class). The

directed towards the rôle played in development by the segregation and localization of cytoplasmic materials. Roux himself ('03) has now abandoned the second of these "Annahmen" (qualitative nuclear division).

earlier results formulated for these types seemed wholly subversive of the mosaic-principle and of the nearly related one of germinal prelocalization. In the sea-urchin egg, the first in which an isolated blastomere was shown to be capable of producing a complete dwarf larva, the experiments seemed at first to show that the blastomeres are composed of indifferent material, so that, to cite an early statement of Driesch's, "Durch die Theilung bei der Furchung völlig gleichwerthige, zu allem fähige (indifferente) Stücke geschaffen werden" ('92.2, p. 36), forming a material "welches man in beliebiger Weise, wie einen Haufen Kugeln durch einander werfen kann, ohne dass seine normale Entwicklungsfähigkeit darunter im mindesten leidet" (*op. cit.* p. 25). Despite the fact that Driesch early recognized that the cytoplasm of this egg is not isotropic, he considered that his experiments definitely overthrew His's principle of "Organbildende Keimbezirke," or at least deprived it of all casual significance in the echinoderm egg ('92.1, p. 178, '93, p. 243). Specification of the early cleavage cells was denied ('92.2, p. 22), as was also the principle of mosaic development as applied to this egg (*l. c.*, p. 36). Again, in the paper of 1899, on "Die Localization morphogenetischer Vorgänge," where Driesch's theory of vitalism is first definitely formulated, the ground is taken that "Darin nämlich, das jeder beliebige Eitheil, sowie das Eiganze in beliebiger Verlagerung eine ganze Larve liefern, also jede 'Organization,' die postulierte Vorbedingung zum Eintritt lokalisirten specifischen Geschehens überhaupt, nach Störung, regulatorisch wieder herzustellen vermag, kommt zum Ausdruck, das eben die 'Struktur' des Eies *nicht* aus mannigfach-verschiedenen Elementen in irgendwie typisch-specifischer Lagerung aufgebaut sein könne, die etwa zu den späteren Differenzierungen in irgend einer Beziehung stünde" (*op. cit.*, p. 43). It is hardly necessary to point out how greatly all this has been changed by Boveri's discovery of the fact that the sea-urchin egg does in point of fact contain "mannigfach-verschiedene Elemente" disposed in a "typisch-specifischer Lagerung," which are proved by the experiments of both Boveri and Driesch to stand in definite relation to the subsequent process of cleavage and differentiation. The relation of



these facts to those determined in the nemertine and mollusk is considered beyond. I will at this point only express my agreement with the conclusion of Fischel, that "Sowohl bei den Echinodermen, wie bei den speciell so-genannten 'Mosaik-eier' erfolgt die normale Entwicklung im Wesentlichen als Mosaik-arbeit" (Fischel, '03, p. 728). I am convinced that had the experimental analysis of cleavage been first undertaken in the case of such a determinate type as that of the gasteropod or annelid, and had Roux not handicapped his theory with a purely speculative hypothesis of differentiation, which proved to be untenable, the whole discussion would have taken a very different course; and I believe it would from the first have been recognized that the mosaic-principle holds true in greater or less degree for every type of development, not excepting the most "indeterminate" forms of cleavage.

My experiments on the unsegmented egg of *Dentalium* have added fresh proof to that obtained by Fischel and his predecessors in the ctenophore, that the cleavage-mosaic is a mosaic of specifically different cytoplasmic materials, in which are somehow involved corresponding morphogenic factors. In this egg, confirming and extending the earlier work of Crampton on the gasteropod-egg, I was able to show even more definitely than has been done in the ctenophore-egg the existence in the unsegmented egg of prelocalized cytoplasmic regions, distinguishable by the eye, that stand in some necessary relation to the formation of the structures to which they give rise in the normal development; for if one of these areas (the lower polar area) be removed, the structures to which it is destined to give rise fail to develop, while if this area remains while other areas are removed the structures in question make their appearance. His's principle of "Organbildende Keimbezirke," which he developed in a purely descriptive sense, is thus shown to have a true causal significance. Since, further, this area contains no nucleus, the conclusion is unavoidable that here, as in the ctenophore—and as we are now able to say, even in the echinoderm—there is a localized distribution to some extent, of the factors both of cleavage and of differentiation in the cytoplasm before development begins. A no less sig-

nificant fact, proved by these experiments in connection with observations by many observers of normal cell-lineage, is that *the germ regions prelocalized in the unsegmented egg are, at least in the case of certain cells, accurately marked off by the subsequent lines of cleavage*. This is shown with great clearness by the history of the lower polar area in *Dentalium* (or the analogous lower green area in *Myzostoma* or the lower polar ring in *Rhynchelmis*), which, although it lies primarily at the center of the lower hemisphere, is not bisected by the first or the second vertical cleavage (despite the fact that both the cleavage furrows first lie exactly in the egg-axis), but is moved to one side so as to pass bodily into one of the cells at each division. Here is an adjustment, of admirable accuracy, by which a specific prelocalized area is handed on from cell to cell, to be finally assigned to its proper position in the cell-mosaic; and if such be the case with one such specific germ area, we have strong ground to infer that it is also so with others. In such cases as these it is evident that the factors of cleavage run so accurately parallel to those of differentiation that they must be referred to a common determining cause, and may be treated as practically identical.

But even in cases where the adjustment is less evident, or less precise (as appears to be the case, for example, in the third cleavage of the echinoderm egg, considered beyond) we shall not, I believe, escape the conclusion that cleavage involves a definite distribution of specific morphogenic factors among the cleavage cells. The facts, proved by my experiments, that these factors may be completely separated and isolated by cell-division, *and may retain their specific character after isolation of the cells*, are only intelligible under the assumption that they are somehow involved in specific materials or stuffs which differ in a definite way and have a specific topographical grouping in the undivided egg. This conclusion is not to be avoided by assuming that the visible cytoplasmic differences are only an accompaniment or consequence of an invisible ulterior structure or organization. Admitting this, and even admitting, for the sake of argument, that the localized cytoplasmic factors are not definitely characterized chemical materials, but only local physical or structural conditions, established

by virtue of the relation of the particular cytoplasmic regions to the egg as a whole: the fact remains that the cytoplasmic substance possesses different specific qualities in different regions, and that these differences persist after the regions have lost their relation to the whole. Only by a play upon words, therefore, can the conclusion be escaped that the cytoplasmic regions consist of specifically different substances having a definite morphogenic value. The question whether these substances are to be considered as preformed building materials, or rather as specific determining materials<sup>1</sup> (such, for example, as enzymes) is a secondary one, on which I do not propose to enter here. Holding both these possibilities in view, I can see no valid objection to the frank adoption, in a provisional sense, of the term "formative stuffs" in the general spirit of the Bonnet-Sachs hypothesis, awaiting future research, to determine what is their mode of action. We must, therefore, conclude that the cleavage-pattern represents literally a mosaic-work of such formative stuffs that have been distributed by the cleavage process, and that the specification of the cells is within certain limits determined by their inclusion of these stuffs. If for the conceptions of qualitative and quantitative nuclear divisions we substitute those of qualitative and quantitative *cytoplasmic* divisions, a very large part of the development that Roux has given to his theory in his long controversy with Driesch, O. Hertwig and other writers, is, I believe, entirely valid. I shall not undertake to go over the whole of this ground again, but will apply these terms to a specific interpretation of certain facts.

In my preceding paper I have suggested that the difference in behavior between isolated blastomeres of different forms is primarily due to differences in the initial form and degree of segregation. The possibility of the production of a perfect larva from either half of any quarter in the egg of *Amphioxus*, *Echinus* or *Cerebratulus* is given by the symmetrical or purely quantitative distribution of materials by the first and second cleavages.<sup>2</sup> In *Dentalium* both halves are not able to produce perfect larvæ,

<sup>1</sup>Cf. Morgan, Regeneration, p. 89.

<sup>2</sup>Cf. Fischel, '03, p. 733.



owing to an asymmetrical distribution of material, the cleavage being visibly qualitative from the beginning; and it is important to note that this asymmetry of distribution is effected by the process of cleavage itself, since the primary segregation-pattern, as far as can be determined, is symmetrical with respect to the axis. In the nemertine or sea urchin the first qualitative division occurs at the third cleavage (which is also qualitative in the mollusk)<sup>1</sup> which for the first time separates ectoblast-stuff from entoblast-stuff. A comparison of the different forms indicates, however, that in respect to this cleavage they differ somewhat in degree. In *Patella* the cells of the first quartet are from the first completely specified, whether as a group or individually, and produce purely ectoblastic embryos that never show any tendency to gastrulate. The same is true in *Cerebratulus*, according to the recent work of Zeleny ('04), which shows that if in the 8-cell stage the upper and lower quartets be separated along the line of the third cleavage, both quartets develop into closed swimming embryos, but the upper one (although the larger) does not gastrulate, though it produces an apical organ, while the lower one gastrulates but produces no apical organ.<sup>2</sup> In the sea urchin, however, a small proportion of the upper cells (20-25%) are able to gastrulate (Driesch, '00. '02.2); and this can only mean that the third cleavage is less strictly qualitative or not invariably so.

<sup>1</sup>As was also assumed by Samassa in the case of the frog's egg, "Diese verschiedenen Entwicklungsbedingungen können aber nur in verschiedenen Substanzen liegen, die bei der qualitativ ungleiche Theilung der dritten Furchung den beiden Zellarten zufallen" ('96, p. 386).

<sup>2</sup>In the light of Zeleny's observations on the 8-cell stage, and in spite of his apparent confirmation of my own preceding results on the blastula stage, it seems to me very probable that the gastrulas I obtained from upper fragments of blastulas in *Cerebratulus* were obtained by slightly oblique section, so that a small group of entoblast cells were included in the upper fragment. I have since observed that the entoblast-plate extends nearly to the equator of the egg, so that even a slight obliquity in the plane of section might give a misleading result. A similar interpretation not improbably may apply to the upper fragments of echinoderm blastulae, cut in two just before gastrulation, which were observed by Driesch ('95) to gastrulate; but these were cut *en masse* without individual orientation, and the experiments evidently do not exclude the possibility that the upper fragments may have contained a part of the entoblast-region. A repetition of this work on both forms by means of individual operation is much to be desired.

Boveri ('01.1) has in fact shown experimentally that the ability to gastrulate depends on the presence of a certain amount of the pigment-band that approximately coincides with the entoblast-zone; and the variation in this regard is explicable under the assumption either of a varying position of the third cleavage-plane with respect to the entoblast-zone or of a variation in the degree of concentration of the entoblast-stuff. While Boveri adopts provisionally the former of these alternatives, he also suggests that the formation of entoblast and mesenchyme is not absolutely predetermined in the plasma, but occurs at the "most vegetative" point, which is the lower pole. Driesch ('02.2) adds the suggestion that the frequent failure of the animal larvæ to gastrulate may be due, not to absolute lack of "vegetativity" ("Um einen nicht sehr schönen aber deutlichen Ausdruck zu gebrauchen"), but to its insufficient degree; and he has recently shown ('03) by an experiment of admirable ingenuity that artificial displacement of the third cleavage-furrow towards the vegetative pole causes a large increase in the proportion of gastrulas produced by the isolated upper cells. This interpretation becomes perfectly intelligible if stated frankly in terms of the formative stuff hypothesis; and it harmonizes with my conclusion regarding the *Dentalium* egg that the influence of the specific stuffs is within certain limits qualitative rather than quantitative, which was based on the fact that if the upper part of the egg be cut away, leaving the whole of the lower pole area the polar lobe typically is reduced to the correct proportional volume, and the resulting larva has a post-trochal region of the proper size. This conclusion is in agreement with that of both Boveri and Driesch, that the plasma structure plays "nur eine determinirende, keine fixierende Rolle" (Driesch, '02.2, p. 522).

In the ctenophore it appears from Fischel's observations ('98) that the first qualitative division is the fourth, which first separates ectoblastic micromere material from the entoblast containing basal cells. In the whole series up to this point we have been considering a segregation that in its initial form is vertical and symmetrical about the axis, though in the mollusk and annelid it becomes asymmetrical in the course of the first cleavage

(proved by direct observation in *Clepsine*, *Rhynchelmis*, *Myzotoma* and *Dentalium*). In the medusæ it appears from the work of Zoja ('95) and Maas ('01), that the primary segregation is not visibly polarized, but concentric; and qualitative division (delamination) does not take place before the fifth cleavage.<sup>1</sup> It seems evident that in these differences of form and degree of the initial segregation pattern we find the leading principle for an explanation of the differences in mode of development shown by isolated blastomeres of the various forms; though as pointed out beyond, a complete explanation is not given by these facts alone. To consider one or two more detailed instances, the first division of the first quartet cells in *Patella* is qualitative, not merely in a descriptive or prospective sense, but actually, as is proved by experiment. By the same standard, the second division of these cells is qualitative in the upper cell ( $1^1$ ), but only quantitative in the lower one ( $1^2$ ). Such facts as these give the strongest ground for the conclusion that all the divisions that would be considered as qualitative or quantitative from the point of view of descriptive cell-lineage, are really such as regards the inherent factors of differentiation. The descriptive and comparative study of cell-lineage represents something more, therefore, than a mere enumeration of successive cell divisions and their geometrical relations, and has the value of a direct examination of the normal morphogenic process.

These conclusions may appear to conflict with the view that has been frequently urged by embryologists in late years that the organism develops essentially as a whole, and that cell-formation plays but a subordinate part in the morphogenic process. The conflict is, I believe, only a seeming one. Roux has repeatedly pointed out that the mosaic-principle is by no means irreconcil-

<sup>1</sup>Cf. Maas: "Wenn die (cytoplasmic) Substanzen in allen Radien, resp. Axen, gleichmässig verteilt sind, wie bei den kugeligen Eiern von Medusen, dann und nur dann hat man ein wirklich *isotropes Ei*; in anderen Fällen, wo ein polare Anordnung festgestellt werden kann, wie bei den echinodermen, besteht die *Isotropie* nur um eine bestimmte *Axe*; in weiteren Fällen kommt durch Gestalt des Eies, wie bei den Cephalopoden, oder durch Lagerung der Substanzen wie bei den Amphibien, eine bilateral-symmetrisch Anordnung zu Stande und in anderen Fällen ist diese Anordnung noch etwas komplizierter (siehe Z. B. *Myzotoma*)." ('03, p. 72.)



able with such a view, and he has steadily maintained the position that the development of every animal presents a combination of self-differentiation and correlative or dependent differentiation, the relation between which varies more or less widely in different cases.<sup>1</sup> Only the most thorough experimental study can determine what this relation is in any individual case. The hypothesis of qualitative nuclear division is no doubt responsible for the disfavor with which the conception of self-differentiation was received by many writers, who either relegated it to a position of quite minor importance or rejected it *in toto*, adopting only hypotheses of correlative differentiation, or advocating a less clearly defined conception of the "organism as a whole," to which the differentiation of the cells was assumed to be subject. O. Hertwig's theory of cellular interaction is a clearly formulated conception of this type, cleavage being assumed to be merely a multiplicative process, producing qualitatively equivalent blastomeres that differentiate by cellular interaction (*e. g.*, '92, p. 481, '93, p. 793). "Die Zellen determiniren sich zu ihrer späteren Eigenart nicht selbst, sondern werden nach Gesetzen, die sich aus dem Zusammenwirken aller Zellen auf den jeweiligen Entwicklungsstufen des Gesamtorganismus ergeben, determinirt" ('98, p. 144). Cell-lineage, therefore, has only an incidental significance, arising from the continuity of development, which involves the derivation of each part from an earlier group of cells, itself in turn the product of a still earlier one ('92, p. 479). Whitman ('93), in his singularly thoughtful and suggestive essay on the "Inadequacy of the Cell Theory of Development," while repudiating the theory of cellular interaction as such, urged with great force the subordination of the individual cells in development to the organization of the embryo as a whole—a conception which, though differing widely in its form of expression, has, I think, much in common with Driesch's theory. The same general view is very specifically interpreted in Child's valuable descriptive paper on the cell-lineage of *Arenicola* ('00), in such statements as the fol-

<sup>1</sup>*Cf.* Roux, '88, p. 455, and elsewhere. Heider, in his suggestive survey of the determination-problem ('00), and Fischel, in his more recent discussion ('03), takes the same ground. See also Korschelt and Heider, '02.

lowing: "The differences between the quiescent trochoblasts and the other cells does not necessarily signify that the former contain a special substance which makes them distinctively trochoblasts from the time of their formation. Of course, at some time they do become distinctly trochoblasts, but simply because of their relation to the whole" (p. 664). I have cited this particular case, since it is precisely in the case of the trochoblasts that experiment most indubitably demonstrates self-differentiation independently of the position of the cell in the embryo. To cite a more general statement, "The material separated as the result of precocious segregation may, I believe, be perfectly indifferent material except as regards position" (p. 682). "Certain amounts, rather than certain kinds of material, are stored up in certain cells just where they will be in position to produce by *coördinated action* the 'desired result'" (p. 679. *Italics mine*). I must own to some difficulty in grasping the conception of a "precocious segregation of perfectly indifferent material"; but, this aside, it is clear that differentiation is considered to be effected, not through the specific and inherent nature of the substance of the individual cell, but through correlative action, the hypothesis even being advanced that an important function of the spiral type of cleavage is to provide for this purpose the most direct and intimate possible communication between the blastomeres (p. 658, etc.).

Lillie, who has contributed such valuable observations on the progressive segregation and organization of the egg-substance, and has recognized in the fullest degree the complexity of that organization and the importance of precocious segregation, nevertheless casts considerable doubt on the conception of prelocalized germ areas ('01, p. 269), and feels constrained to take the position "That the entire organism in every stage of its development exercises a formative influence on all its parts, appears to me an absolutely necessary hypothesis" ('01, p. 273). I do not doubt, as will appear beyond, that this position, with proper qualification, is well grounded; but do not the phenomena of self-differentiation, as shown in the independence of grafts or in the typical differentiation of isolated blastomeres in *Patella*, show that

as thus stated the conclusion is somewhat misleading? I cannot think otherwise. The fundamental conception that the development of every part is conditioned by that of the organism as a whole is one that every embryologist must accept; but it seems to me that Driesch, whom no one will consider a partisan of the mosaic-theory, expresses the truth when he says (*Analytische Theorie*, p. 94), "In diesem Sinne ist nun Selbst-differenzierung *einmal angelegter Teile* ein wesentliches Merkmal der Ontogenese; ja sie ist in Hinsicht auf die spätere Einheitlichkeit und das physiologische Zusammenwirken unaphängig entwickelter Gebilde von einem ganz eigenartigen Interesse" (*Italics original*). The fact must be recognized that the developmental energies and potencies undergo a secondary distribution among the cells or tissues at an earlier or later period, and in varying degrees, which involves corresponding limitations in the secondary centers thus created. We have long been familiar with such limitations in the case of the "germ-layers," though the experimental evidence has shown that they are here less rigid than was formerly supposed. They have been experimentally shown with great clearness by Driesch ('95) in the structures of the blastula, gastrula, and young larva of the echinoderm at successive stages. The experimental results demonstrating the mosaic-character of cleavage have merely shown that similar restrictions of potency may occur still earlier, so as to become manifest even in the early cleavage-cells. Now, it is clear that the primary localization of formative stuffs in the unsegmented egg is essentially an act of the "organism as a whole;" and even though a complete preformation and prelocalization of specific stuffs for every cell and tissue were assumed—and I believe with Boveri and Fischel that such an assumption is not necessary or even probable—we should not escape the necessity for assuming such action of the whole. That the egg undergoes a definite development during its ovarian history and in the stages preceding cleavage, we have evidence both cytological and experimental. The character of the primary segregation-pattern thus determined is indeed determined by the egg as a whole, and the localization thus initiated forms the primary basis of the specification of the blastomeres and organs that de-



velop from the various egg regions. This is quite in harmony with Whitman's contention that "organization precedes cell-formation and regulates it" (*op. cit.*, p. 115). But, while in agreement with the general spirit of his conclusions, as I understand them, it seems to me that Whitman's statement does not sufficiently recognize, first, the fact that the differentiating factors may undergo so accurate and complete a distribution among the cells, and be so largely emancipated from the general control as is proved by my experiments—in other words, sufficient weight is not given to the effects of precocious segregation; second, (and here I should more distinctly take issue with him) that the cytoplasmic segregation or "organization" is a progressive or epigenetic process.

As regards this second point, in my preceding paper I have endeavored to show that the *Dentalium* egg presents a form of precocious segregation (and localization) which in other forms, such as the eggs of certain annelids, is acquired at a later period. The facts observed by Boveri on the *Strongylocentrotus* egg, and the experimental results of Yatsu, Zeleny and myself on *Cerebratulus* clearly indicate that in these forms, too, the cytoplasmic segregation is gradually effected, and at the time of the third cleavage has progressed further in the nemertine than in the echinoderm. There is, therefore, a legitimate basis for the conclusion that the degree as well as the form of segregation existing at the beginning of cleavage may vary more or less widely; and hence for the further assumption that the mosaic character of the early cleavage stages may be expressed in different degrees. For this reason, in so far as the term "organization" as used by Whitman is applied to the cytoplasmic conditions, I am unable to accept his conclusion that the eggs of different forms do not differ in degree of "organization;" or that "Cell-orientation may enable us to infer organization, but to regard it as a measure of organization is a serious error" (*op. cit.*, p. 109). Such a conclusion appears to me a *petitio principii* in regard to the relation between the nuclear and the cytoplasmic organizations, and that between "pre-formed" and "epigenetic" qualities in the cytoplasm;<sup>1</sup> and this

<sup>1</sup>Cf. Boveri, 1902; Wilson, 1904.

question is one to be answered, not by *a priori* considerations, but by observation and experiment. The facts determined by both these methods coincide in showing that the internal factors of cleavage are in a great number of cases so accurately adjusted to the morphogenic factors that they may be treated practically as identical with them. A highly differentiated initial cleavage-pattern is, therefore, *ipso facto* evidence of a high degree of initial cytoplasmic localization; and the fact that the form of cleavage may be artificially altered without affecting the end result is in no manner opposed to this conclusion (as is pointed out in my nemertine paper at p. 455).

I cannot better express the general conclusion which the facts seem to me to justify than by citing the following statement from Fischel's able general discussion ('03, p. 734). "Der Unterschied zwischen den verschiedenen Eiarten ist demnach nur ein *gradueller*, in einzelnen Fällen vielleicht ein *graduell* sehr erheblicher, aber doch kein *essentieller*. Ueberall ist das Grundprincip der (*normalen*) *Entwicklung Mosaikarbeit*, und die besondere Unterscheidung einer Gruppe von Eiern als 'Mosaik'-Eier ist nur in dem Sinne zulässig, als bei ihnen die Mosaikarbeit besonders in Erscheinung tritt; Mosaikeier sind jedoch in gewissem Sinne auch alle übrigen Eier.

"Im Besondern ist aber noch betont, dass wohl stets *nur die Primitivorgane* des Embryo (materiell) in der Eizelle präformirt enthalten sind, und dass—ganz besonders wohl bei den sogenannten Regulationseiern—die materialen Substrate für die Differenzirung der *specialleren* Organe wahrscheinlich überall *erst während der späteren Entwicklungsstadien* gebildet werden.<sup>1</sup> Im Verlaufe der Entwicklung werden stets neue und mannigfache Komplikationen (in erster Linie wohl durch Stoffwechselprocesse, dann durch Lagebeziehungen u. a. m.) gesetzt, durch welche erst bestimmte Zellgruppen des Keims, und zwar vorwiegend durch materielle Umwandlung oder Beimischung oder Differenzirung nach bestimmten Richtungen hin specificirt werden; gerade dadurch aber verlieren sie die ihrem Muttergewebe früher zugekommene Fähigkeit mehr als eben nur jene spezifischen Differ-

<sup>1</sup>Cf. Wilson, '03, p. 453.

*enzirungen gegenbeim Falles zu besorgen, und auf diese Weise gehen Beschränkung der Potenz und Specialisirung für Organbildung einander parallel.*<sup>1</sup> \* \*

"Die hier entwickelte Auffassung betont also gegenüber jener, welche den verschieden Eierten nur eine verschiedengradige 'Regulationsfähigkeit zum Ganzen' zuerkennt, *die verschieden-stufige Abhängigkeit zwischen Organogenese und Eimaterialien*. Die Entstehung von Ganzbeziehungsweise Halbbildungen aus Theilstücken des Eies muss danach nicht auf jene verschieden-gradige 'Regulationsfähigkeit' zurückführt werden, sondern erklärt sich vor Allem aus der Abhängigkeit der Differenzirung von der 'Qualität ihres Ausgangsgebildes' (Boveri), d. h. von dem mehr oder minder vollständigen Schichtenaufbau des betreffenden Eistückes. Der Satz von Driesch 'Jedes Element kann Jedes' ist demnach nur mit dem Zusatze richtig: Vorausgesetzt, dass dieses Element alle zur Bildung des 'Jeden' nothwendigen (im Ei vorgebildet enthaltenen) Plasmaqualitäten besitzt" (*Italics in the original*).

I should only modify the above statement by recognizing the probability that in such extreme mosaic-eggs as those of mollusks or annelids the prelocalization may be much more detailed than Fischel admits, so that for example, the ectoblast may be represented very early in the cleavage, if not at the beginning, not by a single equipotent ectoblast-stuff, but by a number of such stuffs already specified for the production of various categories of ectoblast-cells (trochoblasts, apical cells, etc.). But admitting even to this degree the principles of prelocalization, self-differentiation, and mosaic development, it is still impossible to escape the parallel principle of correlative or dependent differentiation—*i. e.*, the influence of the totality of the organism upon the development of the individual cells. For, however definitely specified a cell or cell-group may be, its behavior when isolated differs in some measure from that shown when in its normal relation to its fellows. The nature of the response to the change of conditions, as the facts show, is, however, conditioned and limited by the factors inherent in the cell or group. The further conclusions are

<sup>1</sup>*Cf. Wilson, '93, p. 610.*



justified, I believe, that these factors differ in different types of eggs from the beginning, and that they become steadily more specialized and limited as the development progresses. With the advance of development, accordingly, the response becomes correspondingly more limited.

This is shown by such a series as the following, including the sea-urchin, nemertine and mollusk. In all these the  $\frac{1}{2}$  or  $\frac{1}{4}$ -blastomere produce an embryo that closes and gastrulates. In the nemertine or sea-urchin any of these embryos may undergo complete development, since the first two cleavages are symmetrical and quantitative, distributing to each cell all the elements of the original system. In the mollusk, however, the AB-half or the A-, B- or C-quadrant, though undergoing certain characteristic differentiations, is unable to produce a complete embryo, owing to the absence of necessary specific material contained only in the D-quadrant or the AB-half.<sup>1</sup> Beyond the 4-cell stage all of the forms exhibit limitations of potency, not primarily due to decrease of size (as is proved by Zeleny's observations on the upper quartet of *Cerebratulus*), but to qualitative internal factors. Cells of the upper quartet, or the entire quartet, produce closed embryos which in the nemertine or mollusk are unable to gastrulate (again owing to the lack of specific material), but in the sea-urchin may do so provided the third cleavage does not exclude a certain amount of entoblast-stuff from the upper cells. The isolated primary trochoblast of the 16-cell stage completes its predestined two divisions and differentiates typically except for slight changes in the relative position of the resulting cells; but the remarkable change of position, which in the complete embryo leads to the accurate fitting together of the rows of cilia; at first disconnected, to form continuous ciliated rings, fails to take place. Its sister-cell (1<sup>1</sup>) likewise divides and differentiates typically; but owing

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<sup>1</sup>I pointed out in the preceding paper that the failure of the CD-half to produce a perfect larva may not improbably be owing to the fact that owing to its great susceptibility, the larva is unable to sustain itself long enough to assume the normal condition. It is theoretically possible that the same may be true of the AB-half; but the actual facts are that the latter shows from the first certain definite defects that do not exist in the former, the CD-larvæ showing merely a lack of the proper proportions.

to the greater number of cells produced, it gives rise to an embryo that closes to form a morula- or blastula-like structure. The single trochoblast of the 64-cell stage of *Patella*, finally, accomplishes no more than a simple rounding out to a spherical form, without undergoing further modification of its predestined development. Each of the reactions in this series of forms must be considered as a response to the change of conditions that results from a destruction of the relation of the part to the whole, and it seems to me the different cases must be considered as differing not in kind but in degree. In any one of these cases the inability to produce a perfect larva is due, as I believe, not to absolute lack of regulative capacity, but to lack of necessary material, which, as far as the experiments show, the cell is not able to manufacture anew; and the degree of regulative response may be considered, other things equal, as inversely proportional to the degree of segregation that has taken place. Only, therefore, in a qualified sense, and in a more or less limited degree, can the prospective value of a cell be considered a function of its position.<sup>1</sup> The sense in which this saying applies to the upper group of four in an 8-cell stage of *Cerebratulus* is far more limited than that in which it applies to a lateral group of four from the same stage (cf. Zeleny). As applied to an isolated primary trochoblast of *Patella*, it becomes so limited as to be largely deprived of its original meaning. The same discrimination is necessary in considering the matter of distribution of potencies in the cleavage-pattern. When, for example, Driesch asserts that "Furchungsmosaik brauch kein Mosaik der Potenzen zu bedeuten" ('99, p. 729, and elsewhere), he is stating a fact that is incontestible as far as the 2- and 4-cell stages of the sea-urchin or nemertine egg are concerned, and which appears to apply to the medusa egg as far as the 16-cell stage; but when in a later paper he advances to the statement, "Furchungsmosaik ist kein Mosaik der Potenzen" ('02, 1, p. 812), an assertion is made that is contrary to the results of experiment, not only on the molluscan egg from the beginning, but even on the nemertine or echinoderm, as soon as the 8-cell stage is reached.

From the facts thus far determined the conclusion seems just-

<sup>1</sup>Cf. Wilson, '93, p. 610.

tified that the power of an isolated blastomere to produce a complete embryo depends upon three conditions: first, upon its volume; second, upon the presence of all the essential elements (and apparently of the cytoplasmic elements) of the system, and third, upon the effectiveness of the regulative process. The production of a complete embryo involves the regrouping of these elements in a disposition essentially like that of an entire embryo, and I see no escape from Driesch's contention that this is a typical act of regulation that cannot be explained without recourse to a factor that lies behind the primary topographical grouping of cytoplasmic stuffs.<sup>1</sup> My observations on *Amphioxus*, the accuracy of which I see no reason to doubt, seem to show that this regrouping may be effected immediately upon the isolation of the cell, as would also seem to be the case in the inverted single blastomeres of the frog's egg observed by Morgan (though observations on the cleavage in this case are still lacking). In the greater number of cases thus far observed the cleavage-factors, and hence as I think we now may say, probably also the morphogenic factors, do not undergo immediate readjustment; and it is still quite an open question to what extent the cells formed in the ensuing partial cleavage undergo changes of prospective potency. But even though all the essential elements of the system be present, in a mass of sufficient volume, a failure of regulation may occur, perhaps owing to merely physical obstacles. As pointed out in my preceding paper, this is not improbably the explanation of the failure of the CD-half in *Dentalium* to produce a perfect larva. The ctenophore-egg is of exceptional interest in this direction; for there is good reason to conclude (since both the cleavage and the larva are bi-radially symmetrical) that the vertical cleavages—i. e., the first and second, and perhaps also the third—are not qualitative, yet, notwithstanding the closure of the embryo produced by the  $\frac{1}{2}$  or  $\frac{1}{4}$ -blastomere, the larva remains defective. Driesch's explanation of a failure of the regulative process owing to a "rigidity" of organization or of protoplasmic texture seems in this case perfectly valid; but such explanation must be consid-

<sup>1</sup>Cf. Lillie, '01, p. 269; Driesch, '02.1, '02.2; Wilson, '03, p. 456.



ered inadequate for the cases of qualitative division reviewed above.

As regards the relation between self-differentiation and dependent or correlative differentiation, our only guide must be the indirect evidence derived from the response of the cell to the change of conditions when its typical relation to the whole is destroyed by isolation or displacement from its normal position. For it is perfectly obvious that if the "atypical" or secondary changes characteristic of an isolated blastomere do not take place in a complete embryo it is because of the relation of the cell to the whole of which it forms a part; and it is this "relation" that renders the developing organism a unit, even in the most highly differentiated type. As to what this "relation to the whole" really is we know practically nothing; but even though we employ a phrase of vague and uncertain content it is of use as indicating a unity or harmony of organization that is not destroyed by the secondary distribution of the factors of differentiation among localized centers.

It is obvious that the differentiation even of such cells as the primary trochoblasts, which possess so high a degree of self-differentiation, must be definitely coördinated in some way with the development of the embryo as a whole, as is shown for instance by the remarkable manner in which the rows of cilia, at first disconnected, are ultimately fitted together to form continuous rings in the prototroch; but it seems equally obvious that in such cases correlative differentiation subsequent to division plays but a minor part in the internal transformation of the cell. I may here point out, however, that the lessened inequality of division so frequently observed in the isolated blastomeres is possibly an indication of regulative response on the part of the internal factors of cell-differentiation. It is clear that the position of the spindle—and hence the character of the ensuing division—is definitely correlated with the segregation-pattern; and in the molluscan egg many, probably all, of the earlier unequal divisions are qualitative in character. It is, therefore, a fair hypothesis that in these cases<sup>1</sup>

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<sup>1</sup>The unequal division of teloblasts shows that the statement should not be made general without further evidence.

the inequality is caused by, or at least correlated with, a preceding segregation of different materials in the cell before division. Hence it is an interesting fact that all the typically unequal divisions of the normal development show a tendency to become less unequal upon isolation of the cell. This has been observed in the first division of the isolated  $\frac{1}{4}$ , of the  $\frac{1}{8}$ -micromeres, of the  $1'$  cells, of the  $\frac{1}{8}$ -micromeres, the  $\frac{1}{16}$ -macromeres, and in the CD  $\frac{1}{2}$ -blastomere of *Dentalium* (where it is expressed by a reduction in the size of the polar lobe), all of which are qualitative divisions. This may be explicable as a result of relatively simple physical conditions, but it is at the same time not improbably an expression of a tendency for the segregation to recede, as it were, towards a less definitely localized condition. The possibility is thus suggested that the segregative process in the cells when in their normal position in the whole embryo may, even in relatively late stages, be in some measure influenced by their relation to their fellows or to the whole. I believe that important light may be thrown on this question by an accurate comparison of the later development of isolated blastomeres that vary in this respect.

The most important question remaining is whether after complete segregation and isolation of specific cytoplasmic stuffs has been once effected by qualitative division the missing materials may be restored by regulative metabolic processes. Such remarkable facts as those determined in regard to the regeneration of the lens in *Triton*, or Spemann's hardly less striking results on the formation of double-headed monsters in the same animal, leave no doubt that specific cell-characters may, within the limits of the germ-layers, be very widely altered through a response to a local defect, or to a change as simple as a mere mechanical alteration of form in the growing mass; and the facts of regeneration even seem to show that one of the differentiated primary germ-layers may produce structures which in the typical development arise from a different layer. If the hypothesis of formative cytoplasmic stuffs be valid there seems to be no escape from the conclusion that in such cases the necessary formative stuffs may be formed anew. But if the potentiality of the cytoplasmic system be primarily given in the nuclear organization, and if this be the

primary determining source of the initial cytoplasmic localization in the unsegmented egg, this presents no insuperable difficulty. It is obvious, however, that this question is one not for speculation but for further experiment.

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March 29th, 1904.

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# CONTRIBUTIONS TO THE PHYSIOLOGY OF THE VENTRAL NERVE-CORD OF MYRIAPODA (CENTIPEDES AND MILLIPEDES).

(Six Figures.)

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## I. *The Rate of Propagation of the Nervous Impulse in the Ventral Nerve-Cord.*

The measurements of the rate of propagation of the nervous impulse were made in the fall of 1902. Their publication has been delayed with the view of obtaining large centipedes from the tropics for the work, so as to exclude a possible source of error in the measurements when smaller specimens are made use of. The attempts to obtain larger centipedes than those available here in California have not proved successful, and the further work must therefore be postponed till more favorable material becomes available.

The structure and relations of the central nervous system of the centipedes and millipedes are essentially the same as in the annelid worms. Each segment is provided with a pair of ganglia, which are connected by transverse commissures and by longitudinal commissures with the neighboring anterior and posterior pairs of ganglia. This nerve-cord is situated ventral to the gut. In the anterior or head segment it is connected by a commissure on either side of the œsophagus with the supra-œsophageal ganglion or "brain."



The method of measuring the rate of propagation of the nervous impulse through this nerve-cord was essentially the same as that employed by Dr. Jenkins and myself in the similar work on the ventral nerve-cord of worms.<sup>1</sup> The preparation and arrangement of the animal for the experiment are shown in the diagram in Fig. 1. The centipede was placed with its dorsal side next to the platform or removable floor of the moist-chamber and se-

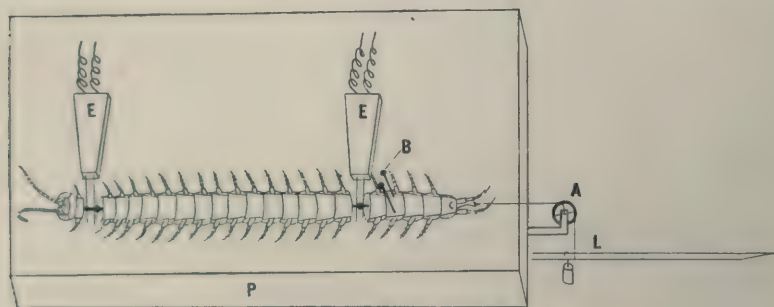


FIG. 1.

Diagram illustrating the method of measuring the rate of propagation of the nervous impulse in the ventral nerve-cord of centipedes. A, friction wheel; B, pins fixing one end of the reacting portion to the platform; E, electrodes; L, recording-lever; P, platform or floor of moist-chamber.

cured to the board by pins, care being taken not to injure the nerve-cord. In *Scolopendra morsitans* and *Scolopocryptops sexpinosus* three or four segments are sufficient as reacting or contracting portion, in the long and very slender centipede *Himantarium taeniopse*<sup>2</sup> eight to ten segments must be used, while in the millipede (*Jules* sp.), in which the actual lengthening or shortening of any part of the body is very slight, eight to ten segments must be employed in order to give sufficient amplitude to the excursion of the recording-lever. The segment next to the reacting portion was fixed to the board by means of two pins in the manner shown

<sup>1</sup>Jenkins and Carlson, *Journal of Comparative Neurology*, XIII, p. 259, 1903.

<sup>2</sup>These centipedes were identified for me by Mr. R. V. Chamberlin, of Cornell University. The centipede *Stylolamius*, sp., made use of in studying the reflexes, was identified by Mr. R. E. Snodgrass, of Stanford University.

in Fig. 1, so that the contractions of the body anterior to this point could not be communicated to the lever. The freeing of the nerve-cord for the application of the distal and proximal electrodes is a very difficult undertaking, and in no instance was it done as completely as indicated in Fig. 1, especially in the slender *Himantarium*, in which the nerve-cord is correspondingly slender, and in the millipede, in which the dissection is rendered difficult by the very thick chitenoid epidermis. The dissection for the proximal electrodes was in every case made at least two or three segments from the reacting portion of the body, to avoid escape of the current directly to the reacting musculature. In *Himan-*

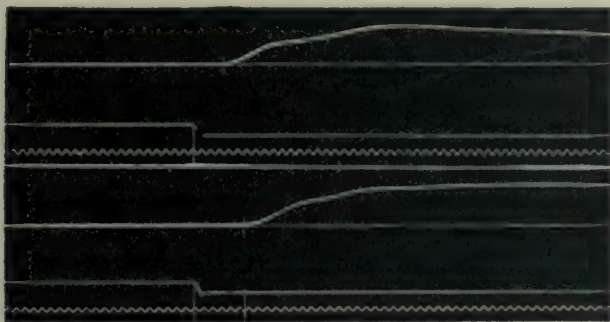


FIG. 2.—*Scolopendra.*

Tracings of the contraction of the posterior segments on stimulation of the cord at the distal and the proximal points. Length of cord, 5cm. Transmission time of the impulse, 0.02 sec. Rate, 2.50m. per sec. Time, 100 d v. per sec.

*tarium* six to ten segments were allowed to intervene between the point of stimulation and the reacting portion.

No anæsthetics were used, but prior to fixing the animal to the platform the head segment, including the cerebral ganglion, was usually removed.

The posterior or tail segments of the decapitated centipede which has been fixed to the board and prepared in this manner usually become quiescent after a few minutes, and remain quiescent during the intervals between the stimulation of the cord, provided the tension from the recording-lever is not too great.

When the tension due to the weight of the lever is considerable the segments are kept in constant motion until exhausted. And the same is true if the segmental appendages or legs are able to reach or touch any object. The contact of the legs with any solid object evidently starts reflex movements of locomotion, and for that reason the preparation does not become quiescent until nearly exhausted when fixed to the platform ventral side down so that the ambulatory appendages are in contact with the board. When the anterior end of the centipede serves as the reacting portion the reflex restlessness is much greater than when the posterior segments are employed. This is true whether the head segment is removed or not. The measurements of the rapidity of conduction of the postero-anterior impulses in the cord by the present method is therefore attended with greater difficulties than the

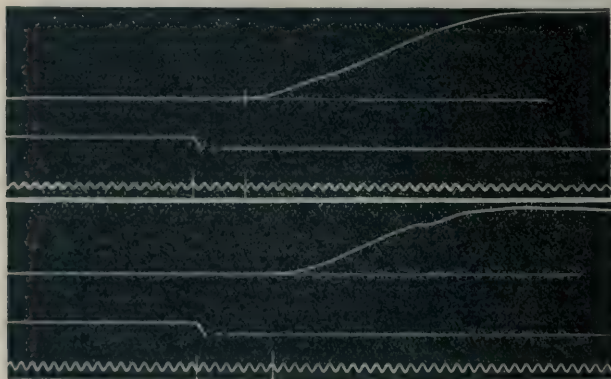


FIG. 3.—*Scolopendra*.

Tracings of the contraction of the posterior segments on distal and proximal stimulation of the cord. Length of cord, 6 cm. Transmission time of the impulse, 0.022 sec. Rate, 2.70 m. per sec. Time, 100 d. v. per sec.

measurement of the antero-posterior rate. In the millipede the union of the segments admits of only slight elongation and contraction of the body, but the body may be coiled by contraction of the ventral muscles in the segments. The amplitude of this contraction is much greater in the posterior than in the anterior



portion of the body, and for that reason the postero-anterior rate of the nervous impulse cannot very well be determined with the present method.

In the centipedes *Scolopendra* and *Scolopocryptops* a single induced shock of moderate intensity applied to the nerve-cord either at the anterior or at the posterior end of the body produces contraction of every segment in the body. In the work on these animals the break induced shock was therefore used as the stimulus. This reaction to the single induced shock is not obtained in the long and slender centipede *Himantarium* or in the millipede. In

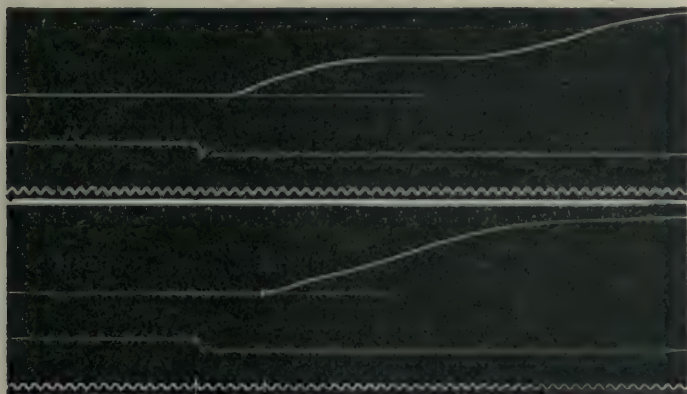


FIG. 4.—*Scolopendra*.

Tracings of the contraction of the anterior segments on proximal and distal stimulation of the cord. Length of cord, 4.5 cm. Transmission time of the impulse, 0.03 sec. Rate, 1.50 m. per sec. Time, 100 d. v. per sec.

*Himantarium* a single induced shock even of very great intensity applied to the anterior or posterior end of the nerve-cord does not always produce a contraction that extends over the whole animal. The contraction extends further from the point of stimulation the stronger the induced shock, but rarely from one end of the animal to the other. When the cord is stimulated with three or four weak induced shocks that follow one another in rapid succession the contraction involves every segment in the body. In the experiments on this centipede short series of the interrupted

current was therefore used as stimuli. A single induced shock applied at one end of the nerve-cord of the millipede *Jules* produces progressive movements of the ambulatory appendages or legs from the point of stimulation to the opposite end of the animal, but the contraction of the muscles moving the body segments is confined to the immediate vicinity of the point of stimulation; but a short series of the tetanizing current produces contraction of these muscles in all the segments of the body. A similar condition was found by Dr. Jenkins and myself to obtain in the marine annelid *Aphrodite*, in which a single induced shock applied to the ventral nerve-cord produced contraction of the muscles that move the setæ, but a tetanizing current was required to produce contraction of the muscles moving the segments. It is therefore probable that the nervous mechanism of the setæ in *Aphrodite* and of the legs in the millipede is less complex and more readily excited than is the nervous mechanism in connection with the muscles that move the segments. If one of the setæ in the worm and one of the legs of the millipede could be used for raising the lever and the rapidity of transmission of the impulse in this nervous mechanism thus measured, it would undoubtedly be found to be several times greater than that in the nervous mechanism to the segmental muscles.

The character of the records produced by the contraction of the reacting portion on stimulation of the nerve-cord may be gathered from the typical tracings reproduced in Figs. 2 to 6. Only the first part of the tracings showing the latent period and the amplitude of contraction is given, as these are the only points with which we are concerned. In the records from the millipede (Fig. 6) the rising curves represent the gradual bending ventrally of the reacting portion, the movements of each segment fusing into one, apparently continuous, contraction. Each stimulation of the cord by a tetanizing current of short duration usually produces but one such movement. The records from the centipedes are more irregular from the fact that each stimulation of the cord usually starts a series of movements or rather contractions and relaxations which may last for a minute or two in the fresh preparations.

Because of the very complex nature of the muscular part of the preparation the character of the curves, that is, the rapidity and the amplitude of the contraction is not a very accurate guide in determining the admissability of individual records. For example, two successive tracings produced by stimulation of the cord at the distal or at the proximal point may show great divergence in the amplitude of the contraction and yet exhibit the same latent period or they may be nearly identical in the amplitude and rapidity of the contraction and yet show a difference in the latent period of from 15 to 25%. The tracings that showed a greater difference in the amplitude of the contractions than is exhibited by the records in Fig. 3 were usually excluded.

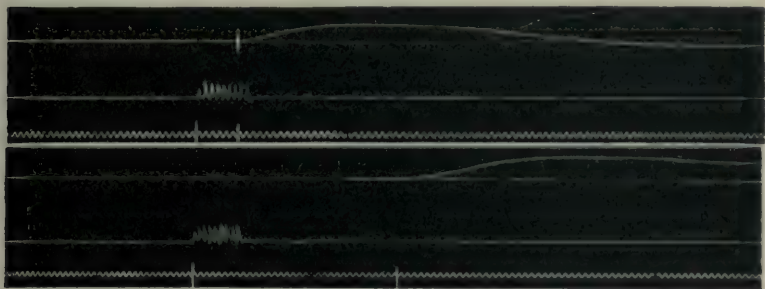


FIG. 5.—*Himantarium*.

Tracings of the contraction of the posterior segments on distal and proximal stimulation of the cord. Length of cord, 14 cm. (120 segments). Transmission time of the impulse, 0.52 sec. Rate, 27 cm. per sec. Time, 50 d. v. per sec.

Of the centipedes worked on the best preparation for these experiments is obtained from *Scolopendra*. The largest specimens yield a length of nerve-cord between the distal and the proximal points of stimulation of from 5 to 6 cm. This centipede is relatively stout and the reacting segments amply able to lift the recording-lever. *Himantarium* is more than twice as long as *Scolopendra*, but is so slender that it is even difficult to fix the specimen to the platform without injuring the nerve-cord with the pins. For the experiments on this centipede the recording-lever had to be very light.



It was stated that the point of application of the proximal electrodes to the cord was always three or more segments distant from the reacting portion. This was done with two ends in view, namely, to prevent escape of the current directly on to the muscle and to prevent errors in the measurements from stimulation of a more direct nervous mechanism on proximal than on distal stimulation. In the annelids the cell bodies of the motor neurones to the musculature of any one segment are situated in the ganglia of the same segment as well as in the ganglia of the adjoining anterior and posterior segments. The conditions are in all probability the same in the nerve-cord of the centipedes and

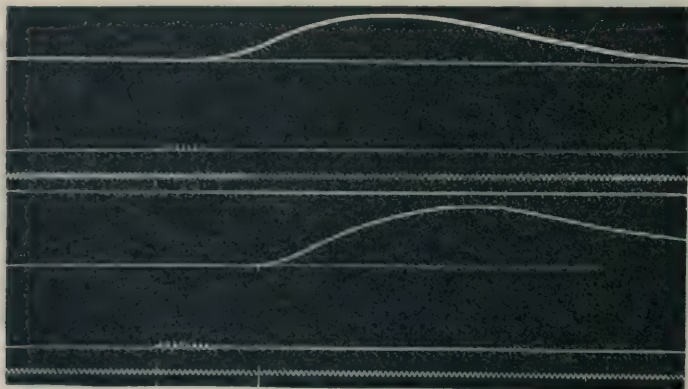


FIG. 6.—*Jules*.

Tracings of the contraction of the posterior segments on distal and proximal stimulation of the cord. Length of cord, 5 cm. Transmission time of the impulse, 0.24 sec. Rate, 21 cm. per sec. Time, 50 d. v. per sec.

the millipedes. Now, if the cord is stimulated in the segment next to the reacting portion it is probable that some of the neurones to the reacting musculature are stimulated directly, while when the cord is stimulated at a point 5 to 14 cm. further away these neurones are probably stimulated indirectly; in other words, there is probably "synapses" at the junction of the longitudinal conducting paths in the cord and the motor cells to each segment. At such junctions the propagation of the nervous impulse is in all probability retarded. If therefore the latent time in the records

on distal stimulation includes this delay while the records obtained on proximal stimulation do not, it is obvious that the rate of propagation of the impulse as calculated from the latent periods of these records would be less than the actual. For that reason it would be desirable to check up the measurements on these comparatively short centipedes by experiments on larger representatives from the tropics, as in larger specimens this possible source of error can be practically excluded.

To give an idea of the variability of the latent time in the records obtained by this method, three series of experiments are given in detail in Tables I, IV, and V. All of the experiments are summarized in Tables II, III, V and VII. The character of the tracings has already been referred to. It is amply illustrated in figs. 2 to 6.

TABLE I.

*Scolopendra morsitans*. Antero-posterior. Detail of experiment No. 2, Table II, October 17, 1902. Temperature, 16° C.

TOTAL LATENT TIME IN SECONDS.

<i>Distal.</i>	<i>Proximal.</i>
0.045	0.028
0.047	0.025
0.048	0.027
0.047	0.026
0.045	0.025
0.047	0.025
Average . . . 0.046	0.026
Transmission time . . . . .	0.02 sec.
Length of nerve-cord . . . . .	5 cm.
Rate . . . . .	2.50 m. per sec.

TABLE II.

Summary of the measurements of the antero-posterior rate in the nerve-cord of *Scolopendra* (No. 1-8) and *Scolopocryptops* (No. 9-13). The length of nerve-cord involves from 13 to 17 segments.

	No. of pairs of records.	Transmission time in sec.	Length of cord in cm.	Rate in cm.
1.....	8	0.020	5	2.50
2.....	6	0.020	5	2.50
3.....	11	0.030	6.5	2.16
4.....	13	0.023	5	2.15
5.....	4	0.025	6.5	2.60
6.....	2	0.020	5	2.50
7.....	3	0.019	6	3.15
8.....	2	0.026	5	1.94
9.....	4	0.015	5	3.33
10.....	4	0.015	4	2.64
11.....	3	0.016	4	2.40
12.....	8	0.017	4.5	2.60
13.....	3	0.024	6	2.46
Mean rate.....				2.50 m. per sec.

TABLE III.

Summary of measurements of the postero-anterior rate in the nerve-cord of *Scolopendra*.

	No. of pairs of records.	Transmission time in sec.	Length of cord in cm.	Rate in cm.
1.....	5	0.040	6	1.50
2.....	4	0.032	4.5	1.40
3.....	4	0.040	7	1.75
4.....	3	0.037	4	1.08
5.....	4	0.040	6	1.50
Mean rate.....				1.40 m. per sec.

TABLE IV.

*Himantarium taeniopse*. Antero-posterior. Detail of experiment No. 2, Table V, November 5, 1902. Temperature 18° C.



TOTAL LATENT TIME IN SECONDS.

<i>Distal.</i>	<i>Proximal.</i>
0.46	0.10
0.48	0.11
0.51	0.13
0.40	0.10
0.42	0.09
0.46	0.11
0.45	0.09
0.45	0.13
0.43	0.13
0.45	0.11
0.46	0.13
Average . . . 0.45	0.11

Transmission time . . . . .	0.34 sec.
Length of cord (100 segm.) . . . . .	10 cm.
Rate . . . . .	26.4 cm. per sec.

TABLE V.

Summary of the measurements of the antero-posterior rate in the nerve-cord of *Himantarium*.

	No. of pairs of records.	Transmission time in sec.	Length of cord in cm.	Rate in cm.
1 . . . . .	9	0.43	12 (100 segm.)	27.6
2 . . . . .	11	0.34	10 (100 segm.)	26.4
3 . . . . .	7	0.46	14 (110 segm.)	29.4
4 . . . . .	8	0.37	12 (115 segm.)	32.5
5 . . . . .	8	0.52	14 (120 segm.)	28.0
6 . . . . .	18	0.49	13 (125 segm.)	27.0
Mean rate . . . . .				28.5 cm. per sec.

TABLE VI.

*Jules* sp. Antero-posterior. Detail of experiment No. 1, Table VII, November 16, 1902. Temperature 16° C.

<i>Distal.</i>	<i>Proximal.</i>
0.40	0.16
0.37	0.18
0.37	0.17
0.36	0.14
0.36	0.15
0.37	0.14
0.39	0.13
0.37	0.12
0.40	0.16
0.40	0.12
0.42	0.16
0.44	0.15
Average... 0.38	0.15

TOTAL LATENT TIME IN SECONDS.

Transmission time.....	0.23 sec.
Length of nerve-cord.....	6 cm.
Rate .....	25.8 cm. per sec.

TABLE VII.

Summary of the measurements of the antero-posterior rate in the nerve cord of the millipede *Jules*. The length of cord involves 32 to 37 segments.

	No. of pairs of records.	Transmission time in sec.	Length of cord in cm.	Rate in cm.
1.....	12	0.23	6	25.8
2.....	15	0.36	6	16.8
3.....	4	0.28	5.5	19.8
4.....	2	0.38	6	16.2
5.....	5	0.35	6	17.4
6.....	7	0.17	5	30.0
7.....	6	0.22	5.5	24.7
8.....	6	0.30	6	20.0
9.....	7	0.29	6	20.4
10.....	2	0.30	5.5	18.1
Mean rate.....				20 cm. per sec.

The rapidity of propagation of the antero-posterior nervous impulse in the cord is the same in the two centipedes *Scolopendra* and *Scolopocryptops*. These two centipedes are also closely alike in the number of segments and in the swiftness of their reactions and movements. The rate is lower than one might have expected, judging by the quick movements of these animals. While it is higher than the rate in the ventral nerve-cord of some of the worms, it is only about one-half that in the nerve-cord of the higher marine annelids *Glycera*, *Eunice* and *Bispira* (one of the *Sabel-lidae*).

The great difference between the rate in *Scolopendra* and *Scolopocryptops* on the one hand and that in *Himantarium* on the other is probably due to a greater number of "synapses," that is, a greater complexity of the conducting path in the cord of the latter. *Himantarium* exhibits a much greater segmental independence than do the other two centipedes. In *Himantarium* the progression of the contraction from the point of stimulation is slow enough to be observed by the eye, while in *Scolopendra* every segment of the body seems to contract at the same time on stimulation of the nerve-cord at any one point. In view of the relatively low rate even in *Scolopendra* it seems to me probable that the conducting path in the cord is not made up of a system of uninterrupted nerve-fibers, although it is evidently less complex than the corresponding conducting path in *Himantarium*.

The rate in the nerve-cord of the millipede is the lowest of all, or only 20 cm. per sec. This is only one-third that of the lowest rate recorded in the nerve-cord of the annelids, namely in the leech (56 cm. pr sec.), and in the marine worm *Aphrodite* (55 cm. per sec.). The reactions and movements of *Jules* are also much slower than those of *Scolopendra* or *Scolopocryptops*. From the fact that the rate of conduction of the impulse in the nerve appears to stand in direct relation to the rapidity of the processes of contraction in the muscle supplied by the nerve,<sup>1</sup> it seems probable that the difference in the rate in *Scolopendra* and *Jules* is not solely apparent and due to the greater complexity of the conducting path in the latter animal.

<sup>1</sup>Carlson, American Journal of Physiology, 1904, IX, p. 401.



A comparison of Tables II and III leaves no doubt that in *Scolopendra* the rapidity of conduction of the impulse through the cord is greater in the antero-posterior than in the postero-anterior direction. A similar condition exists in the case of the spinal cord of the California Hagfish (*Bdellostoma*) and there are indications of the same condition in the spinal cord of the snake.<sup>1</sup> In the annelid *Glycera*, on the other hand, the rate in the ventral nerve-cord is the same in both directions.<sup>2</sup> It is difficult to understand how this difference in the rate of conduction of the postero-anterior and the antero-posterior nervous impulses has come about in the course of development. For the preservation of the individual it would seem that a rapid transmission of the nervous impulse is just as essential over the sensory part of the reflex arch as over the motor part.

## II. *The Reflex Functions of the Ventral Nerve-Cord and the Segmental Ganglia.*

The great difference in the rate of propagation of the nervous impulse in the cord of *Scolopendra* and *Himantarium* lead to the study of the reactions and locomotions of these animals under natural conditions as well as of the reflexes exhibited after severance of the head segment, together with the supra-œsophageal ganglion or "brain," in order to determine whether these animals exhibit other differences in conformity with the difference in the rate of the nervous impulse.

*Himantarium* has two modes of locomotion, namely, by means of its legs and by means of series of contraction waves passing from one end of the body to the other exactly as in the worms. These movements are so identical with those of the worms that the muscular mechanisms are probably the same or at least similar. The centipede works its legs at the same time that it resorts to the other method of getting over the ground. The worm method of locomotion comes into play only when the animal is in a hurry to get away from an enemy. It is made use of with

<sup>1</sup>Carlson, Archiv für die gesammte Physiologie, 1904, C1, p. 231.

<sup>2</sup>Jenkins and Carlson, loc. cit.

equal adaptation in moving either forwards or backwards, just as in the worms. In *Scolopendra* and *Scolopocryptops* the legs are the exclusive means of locomotion, whether the progression is hurried or slow. The chitenoid epidermis attains also a greater development in these centipedes.

*Himantarium* moves backwards or forwards with equal facility and rapidity. When at rest and touched anteriorly it runs backwards; on being touched posteriorly it proceeds forwards. *Scolopendra* or *Scolopocryptops* does not move backwards for any length of time, and never when making haste to escape from danger, as their backward locomotion is much slower than their progression. When *Himantarium* is beheaded its body keeps running backwards continuously for ten to fifteen min. before it starts to move in either direction, while the decapitated *Scolopendra* keeps running forwards, no matter what obstacles are placed in its way, and it is very difficult to induce it to walk backwards, even after the excitation from the injury has partly subsided. It is therefore plain that *Himantarium* and related genera exhibit a less degree of antero-posterior differentiation than do the shorter and stouter centipedes. This is further shown by the fact that when the quiescent *Himantarium*, which is usually coiled up in a bunch, is gently disturbed by light or by touching it, the two ends of the animal will often be found to crawl or move in opposite directions at the same time, that is, the head end walks forwards, the hind end backwards, till the body is straightened out, when either end may take the lead. This was never observed in *Scolopendra* or *Scolopocryptops*.

When *Scolopendra* or *Scolopocryptops* are decapitated by removing the anterior segment, inflicting as little injury as possible to the body, the body usually continues to move forwards incessantly and rapidly for five to ten min., lifting the anterior three or four segments next to the wound high up from the ground. After the elapse of a few minutes the body becomes relatively quiescent, usually moving only when stimulated or touched. If placed on its dorsal side, the decapitated animal straightway turns over on its legs. When the posterior part of the body is touched, it either springs forwards or brings the anterior end of

the body around as if to bite, reactions identically the same as those of the intact animal. When these centipedes are cut in two in the middle the posterior half exhibits the reactions just described, with the exception that it does not turn over on its ventral side so readily when placed on its back, but it attempts to do so in every case. The number of segments may be further reduced without destroying the coördinating mechanism of locomotion. If the sections are made with a razor or a pair of sharp scissors, the whole body may be divided into portions of three or four segments in length, each portion still retaining coördination to the extent that it walks across the table and keeps up locomotion for three to four minutes, but it exhibits no sense of equilibrium—that is, attempting to turn over on its ventral side when placed on its back. The direction of the locomotion in these small portions of the body is almost invariably forwards. The beheaded *Scolopendra* or *Scolopocryptops* live and react in this manner for three to four days. After the initial restlessness, evidently due to the stimulation from the lesion, it scarcely stirs if left undisturbed, although its excitability is retained apparently unimpaired for 24 to 48 hours. It does not react to light. When placed in a glass jar provided with sand or moist earth in one corner it usually comes to rest on these places rather than on the glass.

The beheaded *Himantarium* lives and reacts for seven to eight days, showing much more “spontaneous” activity than the decapitated *Scolopendra*. An 8 to 10 mm. long portion of the body usually exhibits the same reflexes and degree of coördination as the *Scolopendra* deprived of only its head segment. A portion of that length walks forwards or backwards with apparently perfect coordination of its legs, and it turns over on its ventral side when placed on its back, keeping up these reactions for 24 to 48 hours after being isolated from the rest of the body. A portion of three segments walks in either direction, the usual tendency being to forward progression. A portion of five to six segments exhibits the equilibrium reflex in attempting to regain its natural position when placed on its back. Longer portions turn over promptly.



The loss of excitability and death of the decapitated *Himantarium* proceeds antero-posteriorly. When the animal is simply cut in two in the middle the anterior half with the head intact dies sooner than the posterior half. The same is true when this centipede has been bitten in the middle by a *Scolopendra* or a *Scolopocryptops*, in which case the posterior half of the body usually lives for from 12 to 24 hours while the head end ceases to react to stimuli within 2 to 6 hours. The poison of these centipedes is also fatal when introduced into their own bodies. When a *Scolopendra* is seized at its middle by a pair of forceps it usually turns about and bites the forceps, but occasionally it will bite the posterior part of its own body, and always with fatal results, the symptoms of the poisoning appearing in gradual loss of coördination and power of locomotion, death following within 10 to 15 hours.

The decapitated *Stylolaemus* lives and reacts even longer than *Himantarium*, or for 12 to 14 days. The only difference in the behavior of the decapitated and the intact *Stylolaemus* seems to be the absence of the reaction to light in the former. The wounds of the decapitated *Himantarium* and *Stylolaemus* that lived for 8 to 14 days healed in some cases completely. There was no indication of a regeneration of the lost part. The death was probably due to starvation rather than to infection from the wound.

When a number of specimens of *Himantarium* and *Scolopendra* or *Scolopocryptops* are confined together where they can be readily observed, it will be seen that *Himantarium* jerks back and makes haste to get away whenever any portion of its body comes in contact with the other two centipedes. And it has good reasons to do so, as it is an easy prey for these strong and ferocious centipedes. A similar but much less pronounced jerking back of the body is exhibited by all the centipedes studied when they come in contact with the bodies of other individuals of even their own species, especially when the animals are much excited and moving about rapidly, but in no case is it as pronounced as in *Himantarium* on coming in contact with the aforementioned species. The decapitated *Himantarium* exhibits this very same reaction. Especially if the posterior end of the headless body comes in con-

tact with the centipedes, the body jerks back, and both modes of locomotion are usually employed in getting away. That the reaction is more pronounced when the posterior end of the body makes the contact is probably due to reduced excitability of the anterior segments next to the wound. The decapitated animal continues to react in this manner for several days.

The decapitated *Himantarium* retreats from water just as the intact individual, but on coming in contact with other objects in its path it simply walks over or around them. When, however, a solid object, like a pencil or a pair of forceps, is moved towards the crawling centipede and the contact thus made, the decapitated animal usually retreats. When the body comes in contact with an object which is moving towards it, the impact is necessarily stronger than when the object is stationary and the centipede alone moving, hence the difference in the motor reaction is probably due to the *quantitative* difference in the sensory impulses. But the decapitated *Himantarium* jerks back and retreats from *Scolopendra* and *Scolopocryptops* even when these latter lie perfectly dormant, so that the reaction cannot be explained on that ground. One further possibility must be investigated before this reaction can be ascribed to a *qualitative discrimination in the motor reactions to touch impressions on the part of the decapitated centipede*. The touch impressions may namely be supplemented by those of temperature. I have made no measurements of the body temperature of these animals, and until such determinations are made this interesting point must be left undecided.

Cross-section of the ventral nerve-cord in any part of the body destroys the coordination between the two ends of the body on either side of the lesion just as effectively as when the whole body is cut transversely and the two parts rejoined by a thread or a wire. The lesion does not destroy the coördinated locomotion of either half, but the direction of the locomotion of the anterior half may or may not be the same as that of the posterior half. When the direction is not the same, a "tug of war" ensues, in which the portion having the greatest number of segments or having the most favorable ground for contact for its legs comes out victorious. *Scolopendra* usually turns about and bites its refractory hind body repeatedly.

When the millipede *Jules* is cut transversely in the middle the coördination is destroyed in the posterior half. The anterior portion continues to move about for a short time but loss of coördination and death ensue within 10 to 20 min., and the same is true when the animal is decapitated. This animal is therefore not suited for the study of the reflexes and the relative independence of the coordinating mechanisms of the segmental ganglia.

To recapitulate: *Locomotion, movements to regain normal posture, as well as all contact reactions in the centipedes are obviously reflex movements not dependent on the œsophageal nervous complex or "brain," as the decapitated centipede exhibits the same reactions and movements as the intact animal, save that it does not avoid light and cannot feed or make passages for itself in the ground. The decapitated centipede is not abnormally restless, so that any inhibitory functions can be ascribed to the œsophageal nervous complex, nor is it quiescent to the extent that so-called "spontaneous" movements may be said to be wanting. The bending of the anterior part of the body preparatory to bite the object touching the posterior part is a reflex not dependent on the "brain." The maintenance of the body ventral side down is also a reflex through the segmental ganglia, the turning of the body to the ventral side when placed on its back probably depending not so much on the touch impressions on the dorsal side as the absence of the normal touch impressions from the contact of the legs with the ground. The relatively great segmental independence of this equilibrium reflex and especially of the reflex and coordinating mechanisms of locomotion is shown by the fact that these are exhibited by any portion of the body measuring not less than three intact segments in length.*

*The short and stout centipedes (Scolopendra, Scolopocryptops) exhibit a greater antero-posterior differentiation and a less degree of segmental independence than do the long and slender centipedes (Himantarium, Stylolaemus). These latter centipedes retain the annelid mode of locomotion, and the transmission of the nervous impulse through their ventral nerve-cord is slower.*





NOTE ON THE GALVANOTROPIC REACTIONS OF  
THE MEDUSA POLYORCHIS PENICILLATA  
A. AGASSIZ.

BY

FRANK W. BANCROFT.

(From the Rudolph Spreckels Laboratory of the University of California.)

Comparatively few papers on the galvanotropic reactions of coelenterates have been published and so far as I know there are only two bearing directly on the questions here considered. The first is by Pearl<sup>1</sup>, who finds that when any, except the very strongest, galvanic currents are passed transversely through hydra the animal contracts most strongly on the anode side so that the free end—which may be either oral or aboral—swings around and points towards the anode. The tentacles, however, behave differently. With weak currents only those tentacles which are parallel to the current lines contract, but of these the one towards the cathode has a tendency to contract most strongly. When the whole animal has become oriented the tentacles curve slightly so as to become concave on the side towards the cathode. The second observation is by Greeley and will be considered in detail later on.

The tentacles and manubrium of *Polyorchis penicillata*, which occurs abundantly in San Francisco Bay during certain seasons of the year, furnish excellent material for the demonstration of galvanotropic reactions, responding to the current in some respects like the tentacles of hydra, but with greater distinctness. The method of experimentation consisted in cutting the medusæ

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<sup>1</sup>Pearl, 1901, Studies on the Effects of Electricity on Organisms. II.—The Reactions of Hydra to the Constant Current. Amer. Jour. Physiol., Vol. V, pp. 301-320.

in various ways and placing the pieces in a trough of sea water through which the galvanic current was conducted with non-polarizable electrodes. The current strength varied from 25 to 200  $\delta$ . The responses were usually distinct with 25  $\delta$ , but became more decided as the current was increased.

If a meridional strip passing from the edge on one side through center of the bell to the other edge be prepared and the current passed through it transversely, tentacles and manubrium turn and point towards the cathode (Fig. I). A reversal of the current in-

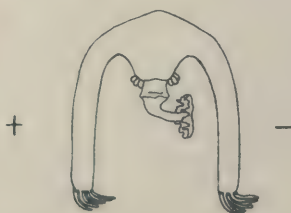


FIG. I.

itiates a turning of these organs in the opposite direction, which is usually completed in a few seconds. This can be repeated many times and the tentacles continue to respond after hours of activity. The manubrium, however, tires sooner and fails to respond. If the strip is placed with its subumbrellar surface upwards and extended in a straight line parallel to the current lines the making of the current causes the tentacles at the anode end to



FIG. 2.

turn through an angle of 180 degrees and point towards the cathode. The tentacles at the cathode end become more crowded together, reminding one of the tip of a moistened paint brush, and also point more directly towards the cathode (Fig. 2). The experiment may be varied in still other ways by cutting smaller or larger pieces from the edge of the swimming bell, but the response is always the same. The tentacles wherever possible, and to a less extent, the manubrium, bend so as to point towards the



cathode. The response depends in no way upon the connection of these organs with the swimming bell, muscles or nerve-ring, for it is obtained equally well with isolated tentacles and pieces of tentacles. Isolated tentacles when placed transversely to the current lines curve so as to assume a more or less complete U-

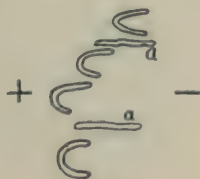


FIG. 3.

shape, with their concave side towards the cathode (Fig. 3). When placed parallel to the current the tentacles do not curve (Fig. 3, a).

If the tentacles are relaxed the making of the current causes them to contract rapidly. Subsequently they turn their concave side towards the cathode, and remain contracted for a considerable period. But if the current is continued long enough through the isolated tentacles a partial relaxation comes on which is suddenly followed by another rapid contraction; so that we have, as this process repeats itself, a slow and irregular rhythmic contraction caused as in the case of the quiescent frogs ventricle by the constant flow of the galvanic current. If the current is continued

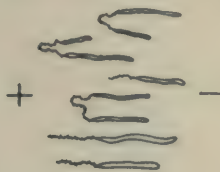


FIG. 4.

still longer in some cases a local anodal relaxation occurs and the isolated tentacles then have the appearance of Fig. 4. As the figure shows, this relaxation is at the bend of the U in the curved tentacles and at the anodal end in those which were parallel to the current lines and did not curve.

It is evident that these phenomena lend themselves very nicely to Loeb's<sup>1</sup> explanation of galvanotropism, which he considers depends on similar changes in the tension of associated groups of muscles. The constant flow of the current brings about an increase of tension on the cathodal side of tentacles and manubrium, as a result of which this part is more strongly contracted than the anodal portion. When the tentacles are exhausted the anodal part may even be completely relaxed.

Greeley<sup>2</sup> has stated that when "*Gonionemus* was exposed to the constant current, rhythmical contractions began always on the cathodal side when the medusa was immersed in normal sea water, but that the contractions began on the anodal side in acidulated sea water." A series of experiments was made on *Polyorchis* to test its behavior in acid and alkaline sea water, but as long as the tentacles were sufficiently uninjured so that they responded at all to the current, they behaved as above described, no matter what the reaction of the water. The influence of acid and alkaline media on the contraction of the muscles was also tested, but Greeley's results could not be confirmed. Usually a change in the reaction of the sea water made no difference, and even when it did the change in the electrical response was sometimes in one direction and sometimes in another, so that no significance could be attached to it.

As a rule the muscles of a meridional strip of *Polyorchis* do not behave towards the galvanic current as described by Greeley for *Gonionemus* in normal sea water; for the place of maximum response is the anode. It is here that the contractions usually start and here that the most rapid rate of the rhythmic contractions is usually seen. But there is such an abundant opportunity for stimulation at secondary cathodes that I am not yet prepared to say that we have here an exception to Pflüger's law.

Berkeley, April 9, 1904.

<sup>1</sup>Loeb, J., 1897. Zur Theorie der physiologischen Licht und Schwerkraftwirkungen. *Pflüger's Archiv*. Bd. 66, p. 440.

<sup>2</sup>Trelcase, W., 1903. Report of a meeting of the Academy of Sciences of St. Louis. *Science*, N. S., Vol. XVIII, p. 753.

# EXPERIMENTS ON THE LOCALIZATION OF DEVELOPMENTAL FACTORS IN THE NEMERTINE EGG.

CHARLES ZELENY.

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The general problem of the localization of developmental factors within the egg has received an important addition in a recent paper by Professor E. B. Wilson, giving strong experimental evidence of a progressive character in the localization of materials in the egg of *Cerebratulus lacteus*.<sup>1</sup>

The present paper is a description of a similar series of experiments on the Mediterranean species, *Cerebratulus marginatus*, carried on at Naples during April and May, 1903, at the sug-

<sup>1</sup>Experiments on cleavage and localization in the nemertine-egg. Archiv. f. Entw. der Organismen. Bd. 16 Heft 3, 1903, pp. 411-460.



gestion of Professor Wilson.<sup>1</sup> The special aim of the experiments was two-fold. In the first place it was desired to throw some light upon the character of changes in localization which take place between the time of fertilization of the egg and the completion of the first cleavage. Since a fragment of the unfertilized egg segments as a whole while an isolated blastomere of the two-cell stage segments as a half, fragments of the egg taken at intermediate stages must yield interesting results. In the second place a comparative study was made of the characteristics exhibited by larvæ developed from different portions of the egg isolated at the eight-cell stage. The three portions thus compared are the upper and the lower quartets obtained by a horizontal cut and the lateral four-cell groups obtained by a vertical cut.

Clear results were obtained on these two points. For the first it is shown that there is a progressive localization of the cleavage factors between the time of fertilization and the completion of the first cleavage. For the second a definite differentiation along the polar axis of the egg is made out at the eight-cell stage. This differentiation occurs in such a way that while a lateral four-cell group remains totipotent, the upper and lower quartets are no longer so, one lacking the ability to form an enteron and the other the ability to form an apical organ.

## 2. *Method.*

The method of operation was a very simple one. The eggs were placed on a glass slide and the water was withdrawn until they were slightly flattened. The cut was made with a fine-bladed scalpel under a dissecting microscope. The resulting parts were then placed in individual dishes, where they were allowed to develop. In fragments of the undivided egg the segments of the sphere thus obtained retained their shape for several minutes, but gradually assumed the spherical form. Fragments of unfertilized eggs were fertilized after the spherical shape had been as-

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<sup>1</sup>I wish to express my great obligation to Professor Wilson for invaluable advice during the progress of the experiments, and to the members of the staff at the Naples Zoölogical Station for continued kindnesses.

sumed. This method of cutting was found to be very successful for segmented eggs as well. Even at the eight-cell stage the upper and lower or the lateral groups of fours may be separated in a considerable percentage of cases without injury to the individual blastomeres notwithstanding the interlocking of the cells.

### 3. Normal Development.

The orientation of the egg in *Cerebratulus marginatus*, as in *C. lacteus*, is made easy by the presence of a basal protuberance before and for some time after fertilization and later by the presence of the polar bodies at the opposite pole. The protuberance is still evident when the first polar body is formed, but usually disappears at about the time of the formation of the second polar body. A considerable difference was noted in the ability to withstand cutting at different periods. Before fertilization the egg could be cut very readily, an extra-ovate being formed in relatively few cases. After the first polar body had been formed, however, the texture of the protoplasm seemed entirely different, the eggs going to pieces in the great majority of cases immediately after the cut was made. Again after the second polar body had been formed the cutting property seemed much better, the quality of the cut resembling that of the unfertilized egg. However, it is very hard to draw any definite conclusion as to the comparative texture of the eggs at these different stages because the amount of flattening of the eggs on the slide, the sharpness of the scalpel and practice in handling the latter may have had a great deal to do with the cleanness of the cut. The general impression, leaving out as far as possible these disturbing factors, is that the protoplasm is much more liquid at the stage with one polar body than that at either the unfertilized stage or the two polar body stage. The maturation divisions seem therefore to be accompanied by a profound change in the nature of the cytoplasm.

When the egg is first removed from the body of the animal there is a large germinal vesicle. This is usually situated on the polar axis of the egg near the side farthest from the basal protuberance (Fig. 3, p. 301). The outline of the germinal vesicle

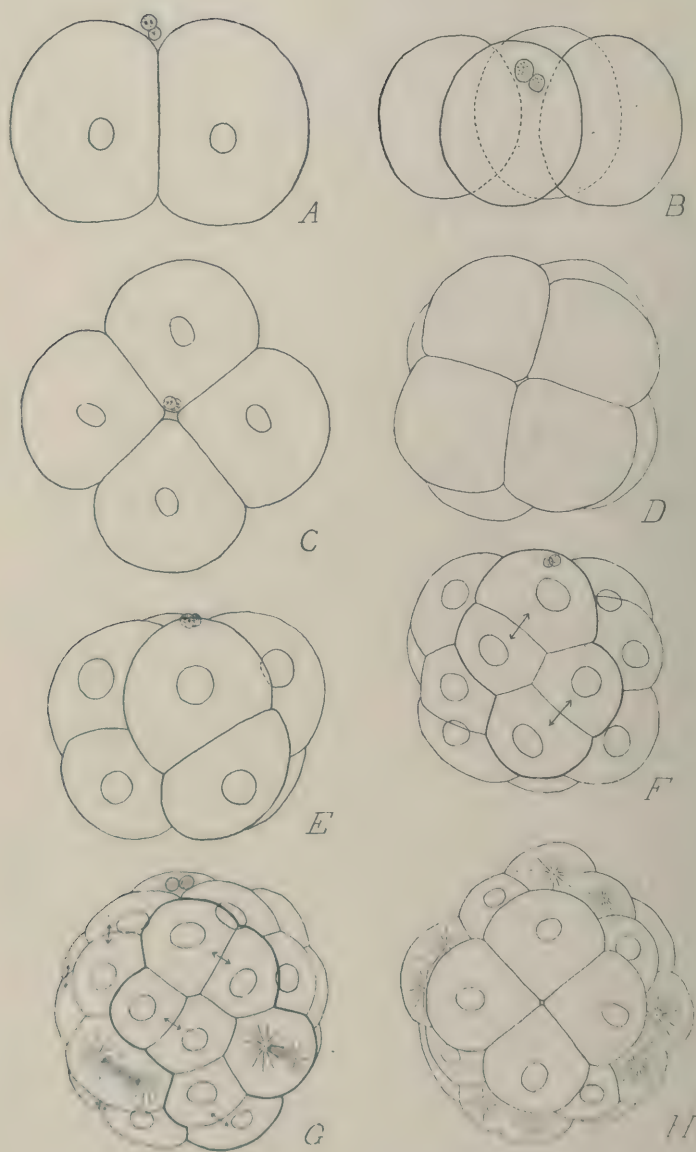


FIG 1 (x 216).



FIG. I.

*Early Cleavage of Entire Egg of Cerebratulus marginatus.*

A, two-cell stage; side view. B, four-cell stage; side view just after the completion of the second cleavage. C, four-cell stage; from upper pole, slightly later than B. D, eight-cell stage; from lower pole. E, eight-cell stage; side view; the relations of the larger upper quartet to the smaller basal quartet are shown in D and E. F, sixteen-cell stage; side view; the outline of a quadrant is indicated by the heavier line. G, twenty-eight-cell stage; side view, slightly from above; the first break in the rhythm of division is shown in the lagging behind in each quadrant of the cell of the intermediate group which had been derived from the basal cell of that quadrant. H, twenty-eight-cell stage; from lower pole.

(The present figures, as well as all the following ones, unless otherwise described, were drawn from preparations with the aid of the camera lucida.)

soon fades away and within half an hour the only sign of it is a clear area which has collected near the pole opposite the protuberance. In this clear area is the spindle of the first polar division. The egg remains in this condition unless fertilized. In the latter case the first and the second polar bodies are formed in succession (Figs. 6, 7 and 9). The cell then elongates, externally a cleavage furrow appears in the vicinity of the polar bodies and later another one at the opposite pole. These constrict the egg into two equal parts. The second furrow, at right angles to the first, also passes through the polar bodies, and the two together divide the egg into four equal parts with no cross furrow or very little indication of one. After the third cleavage, dextro-tropic as usual, the cells of the upper quartet are distinctly larger than those of the lower quartet. The cleavage goes on as a perfect illustration of the spiral type. The cells of the eight-cell stage give out smaller cells by leiotropic cleavages, taking place simultaneously. The further divisions of the cells of the sixteen-cell stage thus formed are not simultaneous. As in *C. lacteus*, the cell of the intermediate group in each quadrant which had been given off by the basal cell of that quadrant lags behind the others, so that there is a distinct twenty-eight-cell stage before the tardy cells divide to form the thirty-two-cell stage. The method of cleavage as here described is very constant for normal whole eggs, variations being extremely rare.

The character of the normal pilidium is too well known to need any description here. The essential features of the early cleavages are given in Figure 1, p. 296, and three stages of the larval development are shown in Figure 2, p. 300.

#### 4. *The Experimental Results.*

a. *Introduction.* The experiments are described here in turn according to the period at which the operation was performed. Ten such periods are recognized:

1. Unfertilized egg.
2. Fertilization to complete separation of the first polar body.
3. First polar body to complete separation of the second polar body.

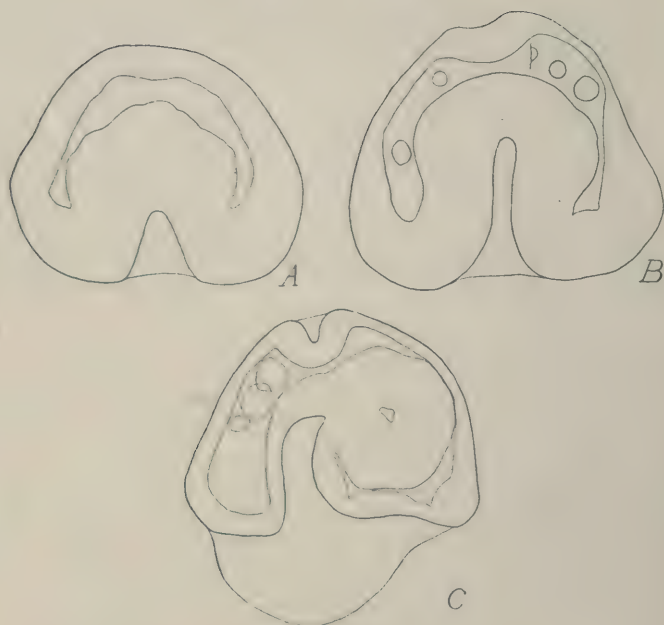
4. Second polar body to beginning of the lateral elongation of the egg.
5. Elongated egg to the completion of the first cleavage.
6. Two-cell stage.
7. Four-cell stage.
8. Eight-cell stage.
9. Sixteen-cell stage.
10. Blastula.

The limits of these periods are fairly well given in their titles and further definition is added later under each head. It may be stated that in every case where the operation was performed after fertilization the sperm had been added to the eggs approximately half an hour after removal of the latter from the animal so that the first polar spindle was already in the metaphase in all cases before the entrance of the spermatozoön. This treatment gave a greater uniformity to the relations of maturation and fertilization than would otherwise have been possible and the external evidences of internal change as given by the polar bodies serve as good landmarks. The various groups of experiments include both the cases observed for the cleavage factors and those for the morphogenic factors. Those on the unsegmented egg were designed mainly to bring out the cleavage factors; those on the two and four-cell stages were intended both for cleavage and morphogenic factors; while those on the eight and sixteen-cell stages and blastulæ were designed wholly for the morphogenic problems.

I. *Fragments of Unfertilized Eggs.* The cuts in this case were made at periods ranging from a half hour to one and a half hours after removal from the mother animal, the eggs being allowed to lie in a dish of sea-water in the interval. Twenty-one specimens were operated on, the cuts being made in the three planes shown in Figure 3. Six of the cuts were horizontal, fourteen vertical and one oblique.

*The localization of cleavage factors* (Figures 4A, B, C). In neither of the three groups was there an indication of a localization of the cleavage factors. The fragments segmented in the regular manner described for normal whole eggs. There is no cross furrow, or only a very short one, and the normal rhythm,



FIG. 2 ( $\times 216$ ).*Normal Larvæ Developed from Whole Eggs.*

A, larva of 23½ hours, showing the beginning of the archenteric invagination. B, larva of 34 hours, showing enteron, mesenchyme cells and apical plate. C, larva of 49 hours, showing apical plate, mesenchyme cells, enteron, and one of the two lappets.

size and position relations are preserved. The only abnormal case is the irregular flat plate of seven cells shown in Figure 4C. When therefore the cases are taken as a whole, the conclusion is very evident that the experiments give no indication of a localization of cleavage factors at this stage.<sup>1</sup>

<sup>1</sup>In connection with the early stages of cleavage the following subsidiary points are to be noted:

1. The nucleated fragments formed polar bodies as in the whole egg, while none were formed in the non-nucleated fragments.

2. No difference can be made out between nucleated and non-nucleated fragments as regards character of the cleavage, each group showing similar features as far as the limited data go.

3. Probable polyspermy, as indicated by multiple division, was shown in two eggs.

4. The direction of the cut has no influence upon the character of the cleavage. Of course, this necessarily follows from the conclusion as stated above, that no localization of cleavage factors is shown in the group.

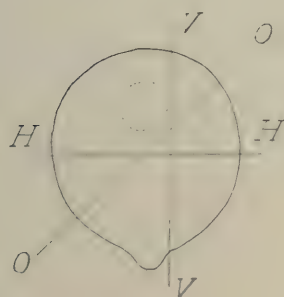


FIG. 3 (x 180).

Diagram of the egg just after removal from the animal, showing germinal vesicle and basal protuberance. HH, VV, and OO indicate, respectively, the directions of the horizontal, vertical and oblique cuts.

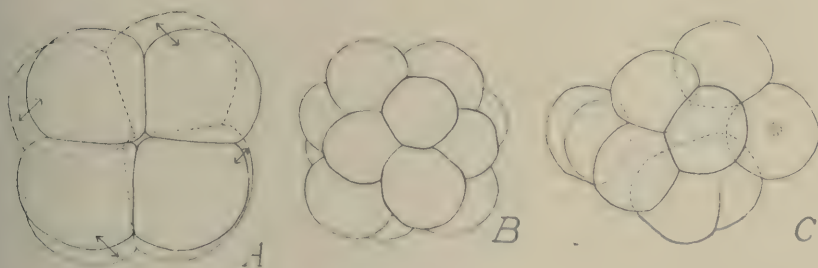


FIG. 4 (x 216).

*Cleavage of Fragments of the Unfertilized Egg.*

A, eight-cell stage, from nucleated fragment ( $=4/5$  of egg), obtained by a horizontal cut; viewed from lower pole. B, sixteen-cell stage, from non-nucleated fragment ( $=3/5$  of egg), obtained by a horizontal cut; side view. C, seven-cell embryo, from non-nucleated fragment ( $=2/5$  of egg), obtained by a vertical cut; viewed from convex side.

*The localization of morphogenic factors.* Only two fragments were allowed to develop into larvæ, and neither of these showed a sufficient differentiation for our purpose. One larva is a solid

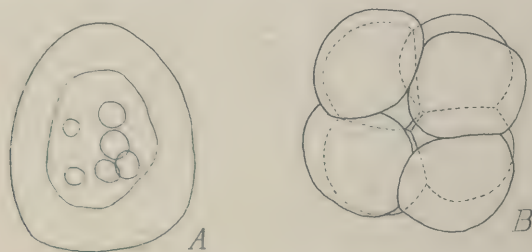


FIG. 5 ( $\times 216$ ).

A, larva developed from a one-half vertical fragment of an unfertilized egg; age 49 hours. B, eight-cell stage of a fragment of a fertilized egg ( $=2/3$  of an egg), obtained by a vertical cut before the formation of the first polar body; except for the unusual cross furrows the cleavage resembles a normal whole one.

ciliated mass of cells and the other, shown in Figure 5A, has an internal cavity with a few free mesenchyme cells.

II. *Fertilization to complete separation of the first polar body.* The limits include the period between the entrance of the spermatozoon and the complete separation of the first polar body (Fig. 6). Seven eggs were operated on, but only two of these developed beyond the two-cell stage. The one clear case bearing on the localization of cleavage factors (Fig. 5B) shows a typical whole cleavage at the eight-cell stage. The morphogenic factors receive no light from the one early blastula obtained.

III. *First polar body to complete separation of second polar body.* The limits of this period are represented by the separation of the first polar body on the one hand and of the second polar body on the other (Fig. 7). Nineteen eggs were operated on, seven by horizontal, ten by vertical and two by oblique cuts. The cases that bear on cleavage factors show in nearly every instance some departure from the normal whole cleavage as regards size, position or division rhythm of cells. The direction of the cut seems, however, not to influence the character of the defect,



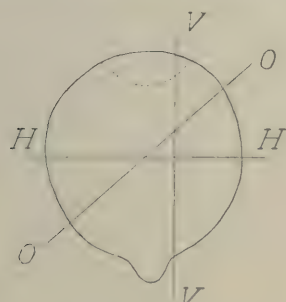


FIG. 6 (x 180).

*Diagram of Egg Soon After Fertilization and Before the Formation of the First Polar Body.*

The dotted line at one pole incloses a clear area containing the first polar spindle. The basal protuberance is still very prominent; HH, VV and OO represent, respectively, the directions of the horizontal, vertical and oblique cuts.

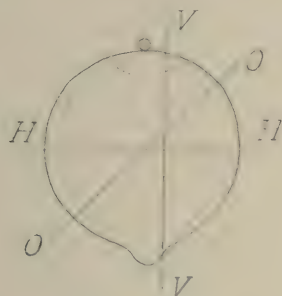


FIG. 7 (x 180).

*Diagram of Egg with One Polar Body.*

The basal protuberance is still evident, but not as prominent as in the former stages; HH, VV and OO represent, respectively, the directions of the horizontal, vertical and oblique cuts.

at least as far as the present experiments go. The most common irregularity is a simple departure from the normal division rhythm (Fig. 8D), which in other cases is accompanied by a displacement of some of the cells (Figs. 8A, B, C). An interesting plate form was obtained from a vertical fragment (Figs. 8E, F, G). Its special interest comes from the fact that notwithstanding the irregularity it formed a swimming larva on the second day, 24 hours after fertilization.

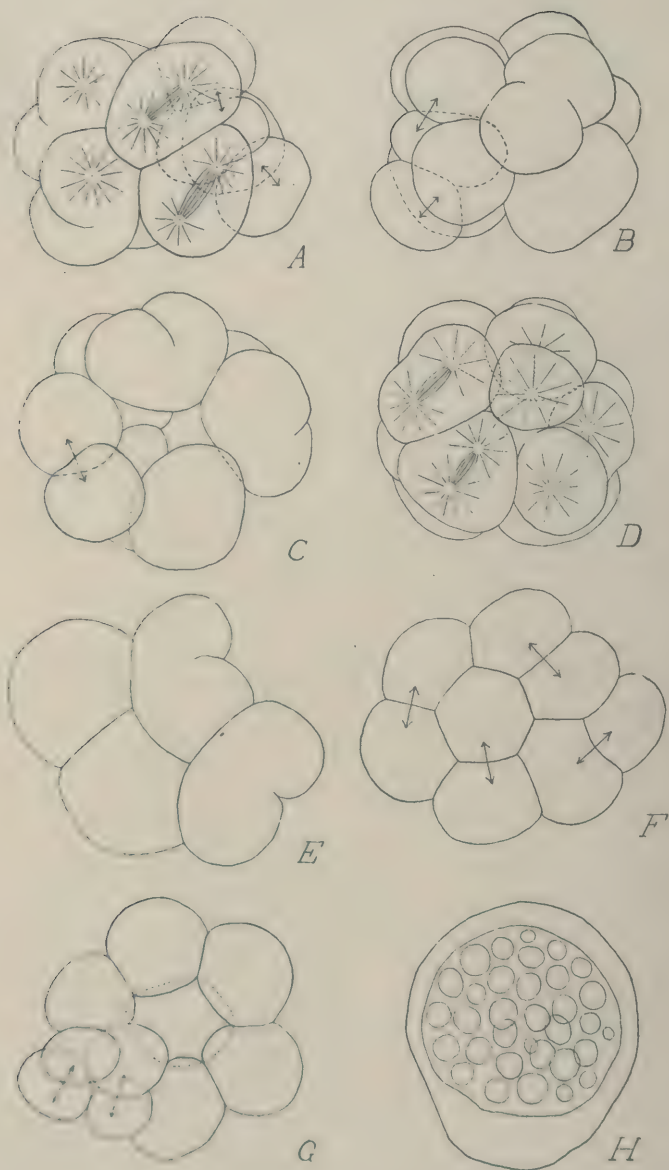


FIG. 8 (x 216).

FIG. 8.

*Fragments Obtained from Eggs with One Polar Body.*

A, 8-16-cell stage of a fragment ( $\approx 4/5$  of egg), obtained by a vertical cut; side view; variations from the normal whole in rhythm of division, in size relations and in position of blastomeres are to be noted. B, side view of same egg from other side after a horizontal rotation through 180 degrees. C, same egg viewed from lower pole. D, 8-16-cell stage of the lower fragment ( $\approx 6/7$  of egg), obtained by a horizontal cut; side view; variations are to be noted in rhythm of division and size relations of cells. Judging by the character of the cut, the fragment probably contained the sperm nucleus without the egg nucleus. E, 4-6-cell stage of fragment ( $\approx 3/5$  of egg), obtained by a vertical cut. F, 8-cell curved plate form of same. G, 10-cell curved plate form of same. H, larva from fragment ( $\approx$  upper  $2/3$  of egg), obtained by a horizontal cut.



None of the three larvæ which were allowed to develop showed a sufficient differentiation of parts to be of service in determining the localization of the morphogenic factors at this stage. The one figured (8H) is a large blastula, the cavity of which is filled with free spherical cells. The other two larvæ, one of which was just mentioned above, both developed after extremely irregular cleavages, and are interesting because they show the presence of an extremely high power of regulation. However, because of the lack of data as regards their structure no definite conclusion can be drawn even here.

IV. *Second polar body to beginning of lateral elongation of the egg.* The period is limited on the one hand by the complete separation of the second polar body from the egg and on the other by the division of the cleavage nucleus and the accompanying elongation of the cell preparatory to the first cell division (Fig. 9). Eleven eggs were operated on, four by horizontal,

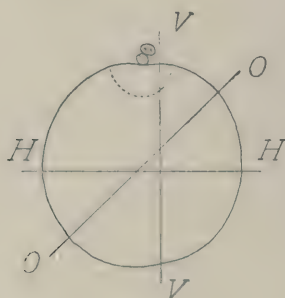


FIG. 9 (x 180).

*Diagram of Egg with Two Polar Bodies.*

The basal protuberance has disappeared; HH, VV and OO represent, respectively, the directions of the horizontal, vertical and oblique cuts.

four by vertical and three by oblique cuts. *The fragments do not segment as wholes, but show very evident departures from that mode.* However, there is a wide difference in the extent of this departure in different cases. As determined by the experiments, the range of the variation is from a possible whole cleavage, through cases with a slight disturbance in size and position of

cells or rhythm of division (Figs. 10A, B, C) up to a case with an open cup-shaped blastula of a purely partial type (Fig. 10E). The results give no definite relation between the position of the removed portion of the egg and the character of the resulting defect in cleavage. A possible instance of such a relation is shown in a sixteen-cell stage developed from a vertical-oblique fragment, which shows a corresponding flattening of one side of the embryo (Fig. 10C).

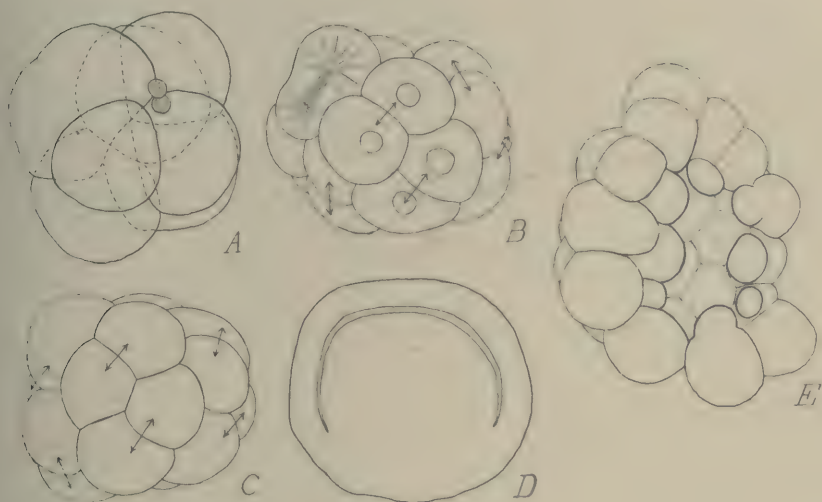


FIG. 10 ( $\times 216$ ).

*Fragments Obtained from Eggs with Two Polar Bodies Before the Lateral Elongation of the Cell.*

A, eight-cell (—) stage of fragment ( $\equiv 2/3$  of egg), obtained by a vertical (slightly oblique) cut; oblique view. Note that the cells of the upper quartet are smaller than those of the lower (the reverse of the normal condition) and that one of the quadrants is behind the others in its division. B, 15-16-cell stage of fragment ( $\equiv 1/2$  of egg), obtained by an oblique cut; side view. Note that the cells differ from normal whole ones in rhythm of division and size relations. C, 16-cell stage of a fragment ( $\equiv 2/3$  of egg), obtained by vertical-oblique cut; side view. Note oblique flattening of egg. D, larva (50 hours old) from fragment ( $\equiv 2/3$  of egg), obtained by a vertical cut; side view. Note solid enteron and absence of apical plate. E, open, partial blastula from a fragment ( $\equiv 2/3$  of egg), obtained by a vertical cut; oblique view from open side.

The localization of morphogenic factors is not particularly elucidated by the two larvæ obtained. The one represented in Figure 10D has a solid interior cell mass, evidently an archenteric ingrowth. There is no other structure sufficiently differentiated for our purpose.

V. *Elongated Egg to completion of first cleavage.* The period is limited on the one hand by the beginning of the lateral elongation of the egg and on the other by the completion of the first cleavage. Five eggs were operated on. In every case the fragments obtained show a partial cleavage from the start, even though in several instances the cleavage furrow was slight at the time of the operation, and there was still a broad connecting band between the two parts of the egg. This band was in every case equal to one-half or more of the diameter of a blastomere of the two-cell stage. The two fragments from one of the eggs are shown in Figures 11A and B at the four-cell stage. It is evident

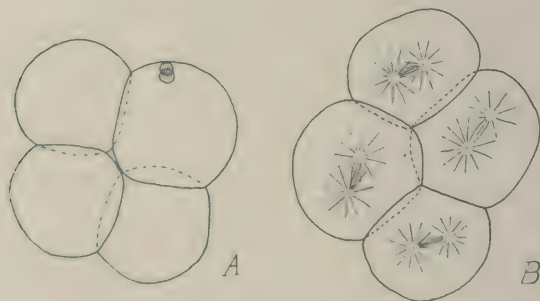


FIG. 11 (x 216).

*Fragments from Eggs Between the Beginning of Lateral Elongation and the Completion of the First Cleavage.*

A, four-cell stage of fragment ( $\approx 1/2$  of egg), obtained by a vertical cut. Polar bodies are attached. The second furrow is equatorial. B, four-cell stage from the other half of the same egg.

that the cleavage is a partial one resembling closely that of isolated blastomeres of the two-cell stage to be described later (p. 309). One of the fragments has the two polar bodies still attached, and it is evident that the second cleavage furrow is equatorial



and not vertical as in the whole egg. The fragments from three of the eggs developed into cup-shaped half blastulæ again, resembling the similar embryos arising from isolated blastomeres of the two-cell stage. There is, therefore, at this period a definite localization of cleavage factors.

As regards the localization of the morphogenic factors no general statement can be made. The two larvæ obtained did not show a sufficient differentiation to be of value.

In the experiments on unsegmented eggs a study of the localization of the cleavage factors has been the main object in view, the few and unsatisfactory isolated observations on larvæ developing from the fragments being incidental and subsidiary to the main point. In the following experiments, however, the study of the localization of the morphogenic factors is definitely taken up, the most extended series and the one yielding the most interesting results being on the eight-cell stage. The experiments on the localization of the cleavage factors are continued for the two-cell and four-cell stages.

## VI. *Two-cell stage.*

1. *Experiments on the localization of the cleavage factors in isolated blastomeres.* The blastomeres were isolated in twenty-eight eggs of the two-cell stage. In the majority both blastomeres segmented, a minority showing no cleavage of one of the parts. In nearly every case the cleavage could be recognized as a partial one corresponding with that of a lateral half of the whole egg. At the four-cell ( $8/2$ ) stage there is a wide cross furrow and the cells are not in the same plane. In fact they appear very much as if they had been removed from the whole eight-celled embryo by a vertical cut (Figure 12A, B). The different forms of cleavage described for isolated blastomeres of the two-cell stage of *C. lacteus* by Professor Wilson were found here also. Their characteristics are especially prominent during the eight-cell ( $16/2$ ) and the sixteen-cell ( $32/2$ ) stages (Figs. 12A to I, 13A). The most numerous are the cup-shaped embryos resembling a geometrical half of a whole blastula of the corresponding age (Figs. 12I, 12H). On the one hand the cups are replaced by

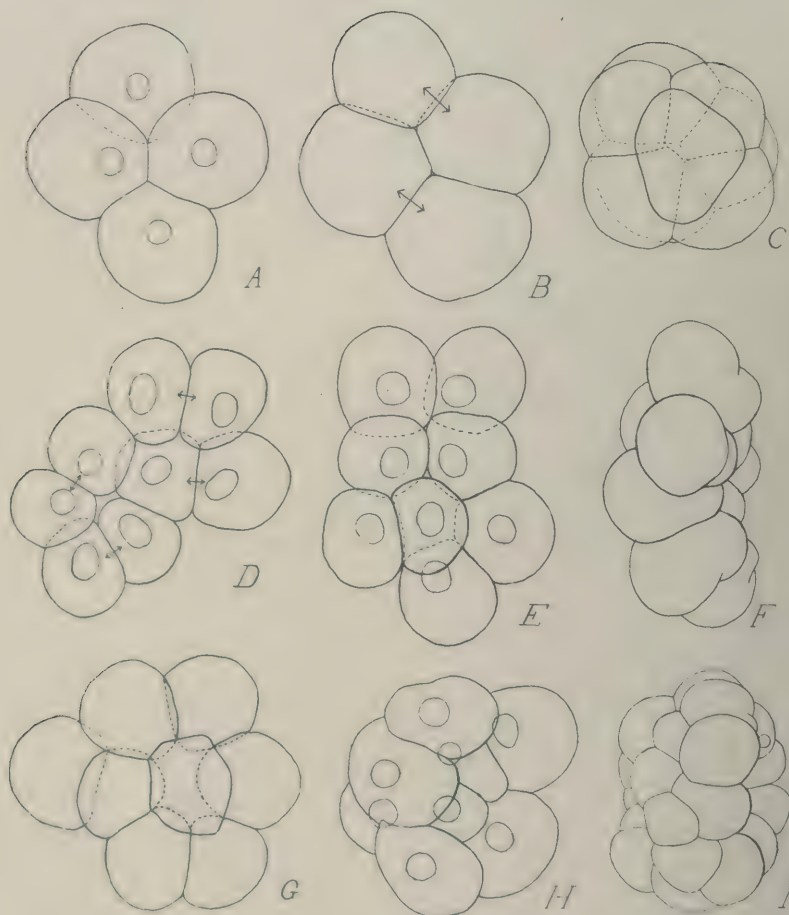


FIG. 12 (x 216).

*Cleavage Stages of Isolated Blastomeres of the Two-Cell Stage.*

A, four-cell stage from an isolated blastomere. Note wide cross furrow; also, that the blastomeres are not in one plane. B, four-cell stage. C, eight-cell stage. D, eight-cell stage of plate type. E, eight-cell stage of slightly curved plate type; viewed from convex side. F, eight-cell stage of curved plate type; side view. G, eight-cell stage of very shallow cup type; viewed from convex side. H, eight-cell stage of shallow cup type. I, 16-32-cell stage of cup shaped type (= geometrical half of normal blastula); side view.

flat plate-like forms, there being all gradations between the embryos curved into a deep cup through those showing only a slight curvature up to perfectly flat plates (Figs. 12D to G and 13A). On the other hand there is a similar graded series from the cup forms up to perfectly closed spherical half-embryos usually containing a very small cavity or none at all (Fig. 12C). It seems probable that these differences of form are the result of slight changes in the surface tension relations between the cells, as Professor Wilson has suggested, and this view is strengthened by my observation of the development of a plate form and a cup form from the two blastomeres of a single egg.

2. *Experiments on the localization of the morphogenic factors.* The localization of the morphogenic factors in the two-cell stage was not made out as fully as could have been wished. The larvæ were killed in most cases at too early a stage to determine the necessary differentiation of organs. The two blastomeres were separated in each of sixteen eggs and in thirteen larvæ were obtained. Most of these were about 33 hours old when killed, only three being older than this. The thirteen individuals are divided into groups of similar cases in the following description.

In *three* cases observations were made on the activity of the embryos, but the embryos themselves were lost during transference to the preserving liquid. An interesting fact in connection with these, and this holds also for other one-half as well as one-fourth embryos, is the abnormally great rapidity of rotation in most of the cases.

Another group is formed by isolated blastomeres from five eggs. The larvæ were distinguished by rapid rotation in life and by a dense ingrowth of cells from one pole, which entirely filled the blastocœle and came close up against the ectoblastic wall around the whole surface of the egg (Fig. 13B, C, D). No apical plate was made out in any of them, but in one case there was a single lappet (Fig. 13B).



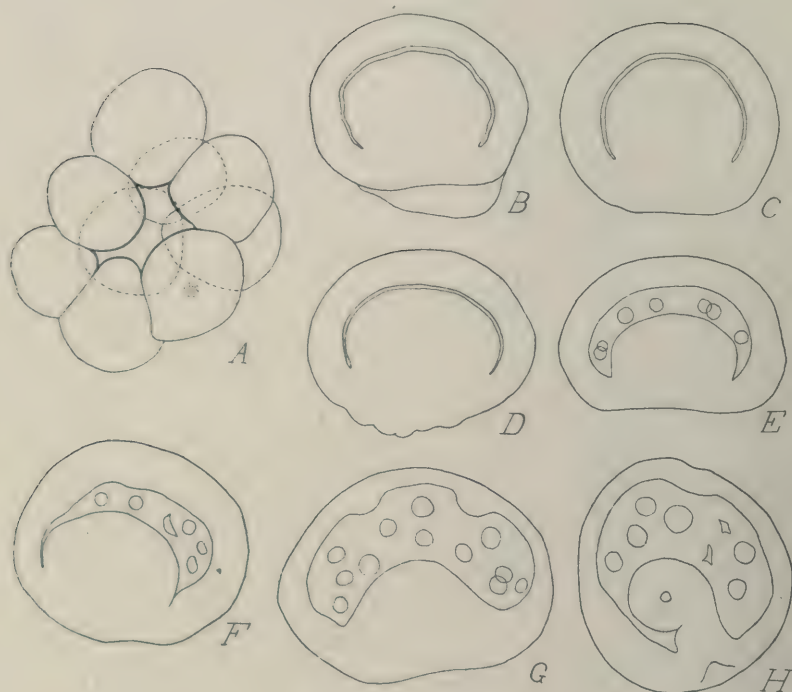


FIG. 13 (x 216).

*Cleavage and Larval Stages from Isolated Blastomeres of the Two-Cell Stage.*

A, nine-cell stage (=cup shaped type); view from concave side. The cut was made at one side of cleavage plane so that the fragment included one blastomere plus part of the other. B, larva (age=33½ hours). Note single lappet, solid enteron and absence of apical organ. C, larva (age=33 hours). Note solid enteron and absence of apical organ. D, larva (age=33½ hours). E, larva (age=33 hours). F, larva (age=33½ hours). G, larva (age=33 hours). The blastomeres were not completely separated and may have fused. H, larva (age=47 hours). Note apical organ and small enteron.

In three cases the embryos resemble the five just mentioned, except that the archenteric mass is not as large and a slight blastocœle, crescentic in vertical section, is present (Figs. 13E, F). This blastocœle contains rounded and irregular mesenchyme cells. There is no apical organ. The larvæ do not differ widely from the normal larva of about 24 hours though their age is 33 hours.

In one of the two remaining cases the blastomeres were not completely separated. The result was *two connected* partial embryos in the early stages, which evidently later fused to form a single individual. The resulting larva (age 33 hours) shows a large blastocœle, two apical organs and a solid enteric mass growing in at the base. The blastocœle contains free rounded cells, and there are no lappets (Fig. 13G).

Finally there is the one case which was allowed to develop for a sufficient length of time (47 hours) to give the organs a chance to differentiate. The resulting larva (Fig. 13H) rotated rapidly in life. It has a large blastocœle, a small enteron, an apical plate and a thickening in the wall at the side of the mouth opening, probably representing the basis of the ectodermal invagination at this point. There are no lappets. With the exception of the small size of the enteron and the absence of the lappets, the larva does not differ widely from a normal larva.

*Summary of the results on the localization of morphogenic factors.* The larvæ developed from isolated blastomeres of the two-cell stage do not show any constant defects except possibly as regards the lappets, organs which in *C. marginatus* are developed at a comparatively late period. Of the instances here cited only two can be considered as old enough to have formed the lappets. At any rate we must consider the larva developed from an isolated blastomere of the two-cell stage to be retarded in development as compared with a normal one of the same age, though this view does not serve to explain completely the characteristics of several of the larvæ.

VII. *Four-cell stage.* The experiments at this period come under two heads. In one series the segmenting egg was divided into two groups of two cells each, and in the other the four blastomeres were isolated.

*The isolated blastomeres segment in every respect as quadrants of the whole egg.* It will be remembered that the whole egg of *Cerebratulus* goes through a definite twenty-eight-cell period because one of the cells of each quadrant lags behind the others in its division as the egg passes from sixteen to thirty-two cells (see Fig. 1G). Correspondingly the isolated blastomere of the four-

cell stage passes through a definite seven-cell (28/4) stage. Such a stage is represented in Figures 14B and C.

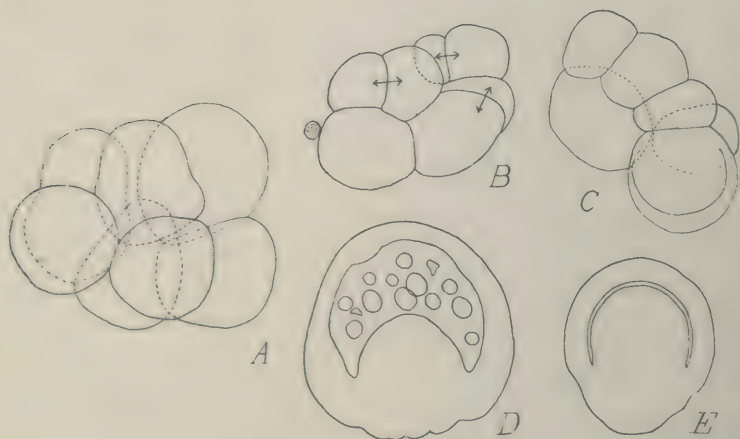


FIG. 14 (x 216).

*Cleavage Stages and Larvæ from Two-Cell Groups and Isolated Blastomeres of the Four-Cell Stage.*

A, seven-cell stage from fragment (two cells+) of egg. B, seven-cell stage from isolated blastomere of an egg. C, seven-cell stage from other isolated blastomere of egg shown in B. D, larva (age=33 hours) from two-cell group. Note apical plate, solid enteric invagination and large blastocœle with numerous free cells. E, larva (age=33 hours) from isolated blastomere. Note the very large solid enteron nearly filling the blastocœle.

Ten eggs were used for the experiments on the localization of the morphogenic factors. The only larva developed from a two-cell fragment was asymmetrical and swam in a small circle. Thirty-three hours after fertilization it had an apical plate and cilia, the beginning of the ingrowth of the archenteric mass and a large blastocœle containing rounded and irregular mesenchyme cells (Fig. 14D). There is thus no definite specification of the morphogenic factors in a two-cell group of the four-cell stage.

In six cases the four blastomeres were isolated, and in five of them very rapidly rotating larvæ resulted. The observations on these were made in most cases thirty-three hours after the fertili-



zation. The larvæ resemble very much the solid larvæ of the isolated blastomeres of the two-cell stage, but are of smaller size (Fig. 14E). All the one-fourth larvæ are like the one figured. There is a solid archenteric growth, but no sign of an apical organ.

The larvæ from isolated blastomeres of the four-cell stage, therefore, give no indication of a definite localization of the morphogenic factors, though they do not develop in an entirely normal manner. The latter statement holds also for the larvæ developed from the isolated blastomeres of the two-cell stage, as has already been stated (p. 311).

VIII. *Eight-cell stage.* The results yielded by the experiments on this stage are perhaps the most important of all those given. The group of experiments included sixty-eight four-cell groups. These groups were separated by a careful cut with the fine scalpel blade used in all the experiments. In most cases the knife blade passed between the cells, and the latter were entirely uninjured by the operation. In a few, however, the protoplasm was cut, and these will be mentioned in the descriptions. The operations include a series of horizontal cuts separating the upper from the lower quartet, and a series of vertical cuts separating the two lateral four-cell groups, each of the latter containing two cells of the upper and two of the lower quartet. There are thus three kinds of four-cell groups, the larvæ from which are to be compared: (1) Upper quartets, (2) lower quartets, and (3) lateral four-cell groups. The experiments yield a very definite and positive result. *The larvæ developing from the upper quartet have an apical organ, but no archenteron, those from the lower quartet have an archenteron, but no apical organ, while those from lateral four-cell groups have both apical organ and archenteron.*

The natural conclusion to be drawn from these results is that certain organ-forming materials are definitely separated by the third cleavage plane, and the larvæ developing from the lower or the upper quartet have not the power of making up the lacking material. The lateral four-cell groups, however, possess both kinds of materials and are, therefore, able to develop both archenteron and apical organ, though the larvæ are usually asymmetrical.

Some of the larvæ are shown in Figures 15, 16 and 17, and it will not be necessary to describe the individual cases in detail, as the results are very definite and clear. The figures give characteristic types of larvæ developing from the upper quartet (Fig. 15), from the lower quartet (Fig. 16), and from lateral four-cell groups (Fig. 17A, B, C). Figure 17D shows a case in which six of the eight cells were represented, two of the lower quartet having been destroyed.

IX. *Sixteen-cell stage.* Five eggs of the sixteen-cell stage successfully withstood an operation, and larvæ from three of these were studied.

In one egg equal upper and lower portions were obtained by a horizontal cut, but there was not a separate identification of them, and they were placed in one dish. At forty-eight hours after fertilization both resultant embryos were ciliated. They showed a difference in that one had ragged edges and swam in a circle, while the other had even edges and remained stationary. The embryos were lost.

In two cases the upper four cells were successfully separated from the lower twelve. The two cases are taken up in turn. In the first one the division was very clear without injury to any of the cells. At forty-six and a half hours after fertilization the upper four cells had formed a small distinctly outlined spherical ciliated embryo, with no rotation or forward motion of the body. There is a distinct blastocœle containing rounded cells, a large apical organ and no enteron or lappets (Fig. 18C). At the same time the lower twelve cells have formed a ciliated rotating embryo, with a large solid archenteron entirely filling up the cavity of the blastocœle. Neither apical organ nor lappets are present. The two embryos thus show a very pronounced difference, the one formed from the upper four cells containing an apical organ and no archenteron, and the other, from the lower twelve cells, containing an archenteron and no apical organ.

In the remaining case the upper four cells were separated from the lower twelve as before. One cell in the former was slightly injured, but all the cells of the latter were left in good condition. From the upper four cells at forty-six and a half hours after fer-

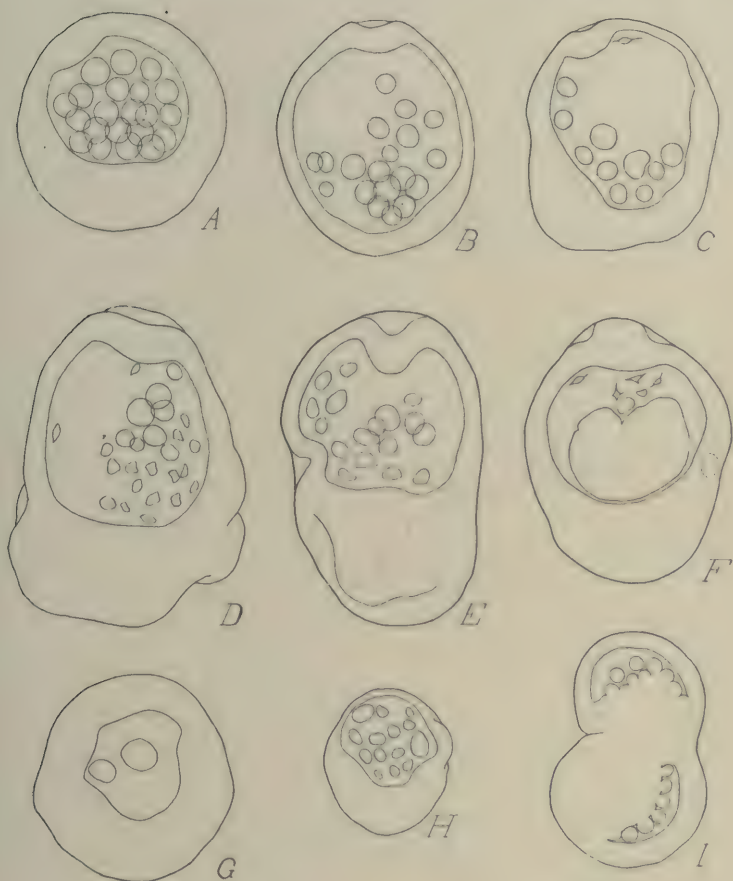


FIG. 15 (x 216).

*Larvæ from Upper Quartets of the Eight-Cell Stage.*

A, larva (age=44 hours) from complete upper quartet. B, larva (age=45 hours) from upper quartet, with one cell injured. C, same larva rotated horizontally. D, larva (age=45 hours) from complete upper quartet. The egg already showed the cell constrictions for the next (16-cell) division. E, same larva rotated horizontally. F, larva (age=45 hours) from complete upper quartet. The inner cell mass does not connect with the side of the larva (*i. e.*, it is free). G, larva (age=23 hours) from complete upper quartet. H, larva (age=33 hours) from isolated blastomere of the upper quartet. I, double larva (age=46 hours) from upper (?) quartet. Note presence of apical organ and absence of enteron in Numbers A to F.



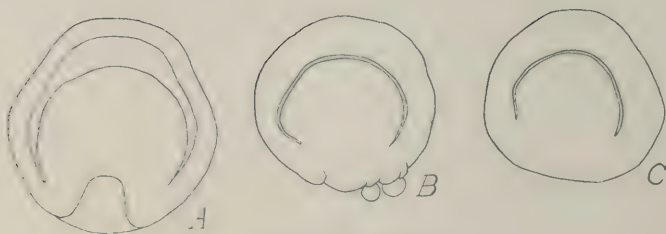


FIG. 16 (x 216).

*Larvæ from Lower Quartets of the Eight-Cell Stage.*

A, larva (age=46 hours) from lower (?) quartet. Note large enteron and absence of apical organ. B, larva (age=46 hours) from lower quartet. C, same larva rotated horizontally: Note solid enteron and absence of apical organ. Note large archenteric ingrowth and absence of apical organ in all cases.

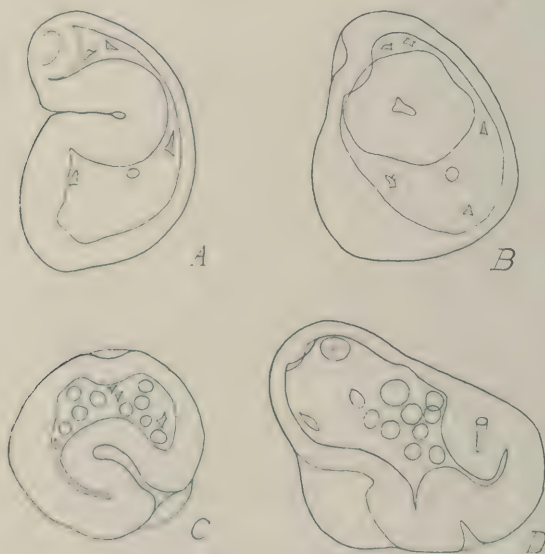
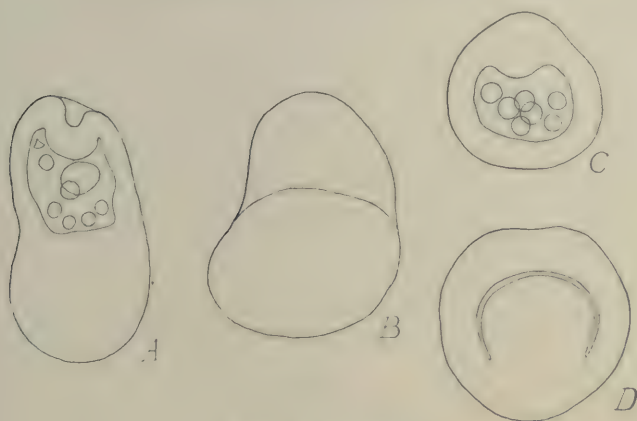


FIG. 17 (x 216).

*Larvæ from Portions of the Eight-Cell Stage.*

A, larva (age=48 hours) from lateral four-cell group. Note presence of both enteron and apical organ. B, same larva rotated horizontally. C, larva (age=33 hours) from lateral four-cell group. Note presence of both enteron and apical organ. D, larva (age=48 hours) from upper quartet plus two cells of lower quartet. Note three apical organs, large blastocoele, small enteron and two ectodermal invaginations at sides of enteron.

FIG. 18 ( $\times 216$ ).*Larvæ from Portions of the Egg of the Sixteen-Cell Stage.*

A, larva (age= $46\frac{1}{2}$  hours) from the upper four cells (one injured). Note presence of apical organ and absence of enteron. B, larva (age= $46\frac{1}{2}$  hours) from the lower twelve cells of the same egg. Note that egg is a solid mass with division into ectodermal and endodermal cells. C, larva (age  $46\frac{1}{2}$  hours) from upper four cells. Note presence of apical organ and absence of enteron. D, larva from lower twelve cells of the same egg; same age. Note solid enteron and absence of apical organ.

tilization an elongated very actively swimming larva with a long apical cilium had developed. The embryo showed after staining and mounting a well developed apical plate. The anterior end of the body is occupied by a blastocœle containing a few scattered free cells. The posterior end is a dense mass of cells, with no signs of an ingrowth of these to form an archenteron (Fig. 18A). From the lower twelve cells at the same time there was developed a ciliated elongated embryo, with only a slight rotation, and no forward movement of the body. The embryo is a solid mass of cells, the only differentiation visible being a difference between the cells at the two ends. Those near one end have the typical histological endoderm characters of the normal larva. while those near the other end have ectoderm characters (Fig. 18B).

The characters of the two larvæ in this case again show the presence of the apical-basal differentiation described for the last

specimen. The experiment seems to indicate that the basis of the apical organ is found in the four upper cells of the sixteen-cell stage. In connection with this result Yatsu's observations on the unsegmented egg of *C. lacteus* are interesting. He localizes the basis of the apical organ in a broad band just above the equator of the egg.

X. *Blastula stage*. Successful operations were made on three blastulæ.

The first one was divided by a horizontal cut into an upper part ( $=2/3$  of blastula) and a lower part ( $=1/3$  of blastula). The orientation was made certain by the presence of the polar bodies. The upper part broke up into two portions, each of which at twenty-four hours had developed into an embryo with an apical cilium. At the same time the embryo from the lower one-third of the blastula was ciliated but had no apical organ. At forty-seven and a half hours the embryo from the lower one-third and one of the upper ones were dead. The other upper embryo has two apical plates, one a well developed and the other a small one, an invaginated ectodermal sac, a large and well developed enteron, a blastocœle with free cells in its cavity, and no lappets. In fact, except for the absence of the lappets and the presence of *two* apical organs, it has all the characters of a typical whole larva (Fig. 19C; only one of the apical plates is shown). However, at twenty-four hours, as stated above, there is a distinct difference between the upper embryos and the lower one because of the presence of the apical organ in the former and its absence in the latter.

A second blastula was cut into equal upper and lower parts by a horizontal cut, but the two were not kept separate. One of the halves died. The other developed all the organs of the normal pilidium, except the lappets. There is a large blastocœle, two apical organs, one in the normal position and one asymmetrically placed and not shown in the figure, and a large long enteron straighter than in the normal larva (Fig. 19B).

A third blastula was cut into two unequal parts equal respectively to two-thirds and one-third of the blastula, by a cut of unknown direction. One portion, the larger one judging by the



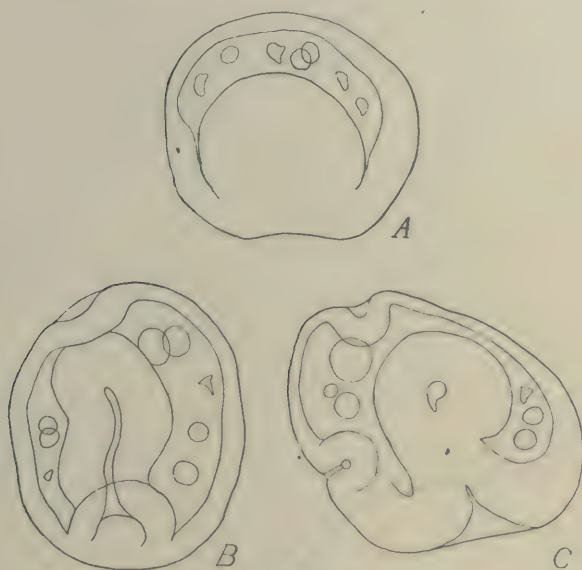


FIG. 19 (x 216).

*Larvæ Developed from Blastula Fragments.*

A, larva (age  $35\frac{1}{2}$  hours) from a fragment ( $=\frac{2}{3}$  of blastula); direction of cut is not known. B, larva (age  $35\frac{1}{2}$  hours) from upper or lower half of blastula. Note presence of both enteron and apical organ. C, larva (age  $=48$  hours) from the upper  $\frac{2}{3}$  of blastula. Note presence of both enteron and apical organ.

size, was the only one alive thirty-five and a half hours after fertilization. Its cilia were waving, but there was no motion of the animal as a whole. The body is spherical, with a large solid enteron nearly filling the cavity of the blastocœle. The chief defect is in the absence of the apical organ, as it is too early ( $35\frac{1}{2}$  hours) for the lappets to appear (Fig. 19A). The direction of the cut is not known and the defect, therefore, cannot be correlated with any definite portion of the blastula.

The experiments on blastulæ give only one organ which can be considered as definitely specialized. The apical plate is developed in each of two embryos from the upper two-thirds of a blastula, while it is absent in those developed from the lower one-third. No explanation can be given of the apparently greater regulative

power of blastula fragments as compared with those of the eight-cell and sixteen-cell stages. A similar fact was noted by Professor Wilson for *C. lacteus*, and he supposes that a possibility of error in orientation of the blastulæ may account for the result. For this reason I took special care in determining the orientation, and in two of the three cases I think there is little doubt of the correctness of the determination.

### 5. Summary of results.

1. Both nucleated and non-nucleated fragments of the unfertilized eggs of *Cerebratulus marginatus* segment as wholes.

2. Isolated blastomeres of the two-cell stage segment as if the other blastomere were still in its place, *i. e.*, they segment as vertical halves.

3. Fragments obtained during the stages between the fertilization of the egg and the completion of the first cleavage show a progressive specification of the cleavage factors as evidenced by abnormalities in rhythm of division, size relations of cells and position of cells. After the separation of the cleavage nuclei and when the cytoplasm of the two cells is still widely connected, the two halves when cut apart may already show all the characters of half cleavages.

4. Isolated blastomeres of the four-cell stage segment as fourths of the whole cleavage pattern.

5. Larvæ developed from the upper quartet of the eight-cell stage always possess an apical organ and lack an enteron, those developed from the lower quartet always possess an enteron and lack an apical organ, while those developed from lateral four-cell groups containing two cells of the upper and two cells of the lower quartet always possess both apical organ and enteron.

6. Larvæ developed from the upper four cells of the sixteen-cell stage lack an enteron, but possess an apical organ and blastocæle. Those developed from the lower twelve cells have a large enteron, but no apical organ or blastocæle.

7. Two embryos developed by a secondary division from the upper two-thirds of a blastula both developed apical organs. The

embryo developed from the lower one-third of the same blastula developed no apical organ.

#### 6. General discussion.

The points brought out by the present experiments are of considerable general interest. In the first place in agreement with the results of Professor Wilson on *C. lacteus*, it is found that while an egg fragment of an unfertilized egg segments as a whole an isolated blastomere of the two-celled stage segments as a half. In the intermediate stages there is a gradually increasing departure from a whole cleavage in the fragments as we pass from the first mentioned stage to the latter. This is contrary to the statement made by Yatsu in his recently published paper on *C. lacteus*. In principle, however, it agrees with the progressive increase of defects found by him in larvæ developing from fragments taken at similar stages.

Though the observations naturally suggest the view that there is a progressive localization of materials in the egg from one period to the other, such a conclusion does not necessarily follow from the experiments themselves without further data. Because, considering the power of regulation of the embryo shown at all stages studied, it must be admitted that there remains the possibility of regulation of the unfertilized fragment to form a complete whole cleavage and later a complete larva. For the earlier the operation be performed the greater the time which must elapse before the fragment divides, and consequently the greater the chance for regulation to a whole cleavage pattern. The experiments of Schultze on inversion of whole frog's eggs at the two-celled stage and the corresponding ones of Morgan on the isolated blastomeres of the same stage, show that the rearrangement of materials due to difference in specific gravity gives opportunities for regulation to a whole development. Observations on the normal eggs of a great many animals during the maturation period show a very extensive series of streaming movements in the protoplasm at this time. May not these furnish a similar opportunity for regulation to a whole cleavage and whole development? For



as the materials in the isolated blastomere of the frog's egg are undoubtedly specialized so as to form a half cleavage pattern and a half embryo under the ordinary conditions, the re-adjustment of materials due to inversion gives the necessary conditions for regulation. May not the unfertilized egg of *Cerebratulus* likewise show a localization of formative factors so that a fragment is a true portion of a mosaic, but needs only the conditions accompanying the streaming during the maturation stages to accomplish a readjustment to a whole? Evidently there is no means of determining this point, because if we assume the possibility of a readjustment during the maturation stages, we remove our only hope of a direct method of deciding the question as to the presence or absence of developmental specification before fertilization. The only remaining method lies in indirect inferences from the observed localization of visible materials during these stages. There is abundant proof of such a progressive localization during this time, and the conclusion that there is an arrangement of formative materials into a definite pattern at this period is a natural one. For there can be no valid objection to the association of the two parallel processes of localization of visible materials and of resultant cleavage and morphogenic factors.

But why is there a progressive localization of the morphogenic factors in the unsegmented egg, as indicated by Yatsu's work, while at the same time the isolated blastomere of the two-celled stage develops into a perfect larva? The progressive localization of cleavage factors as shown in my experiments is naturally to be expected, since there is a gradual passage from a whole cleavage on the one hand to a half cleavage on the other. We may assume a gradual localization of materials controlling these factors, or a greater opportunity for regulation in the earlier as compared with the later stages, or both, to account for the data. With the progressive localization of morphogenic factors, as described by Yatsu, there is no such sequence. Starting with complete larvæ developing from the fragments of unfertilized eggs, there is a gradual increase in the defects in the larvæ up to the completion of the first cleavage. Then very suddenly, as soon as the cleavage is completed, there is a return to whole larvæ. I

think the apparent contradictions may be explained in the following way, though the purely speculative character of all the discussions is always to be kept in mind.

Numerous recent observations, especially those of Lillie and Conklin, indicate that cleavage is an accurate means of separating materials already localized. My experiments on the eight-celled stage of *Cerebratulus* show that the first localization of materials is in an apical-basal (polar) direction. It is probable, therefore, that at the four-cell and even at the two-cell stage this same tendency is the predominant one, so that at the four-cell stage there are four equivalent parts and at the two-cell stage two equivalent parts. However, in each of these two parts (taking the two-cell stage as an example) there is an apical-basal differentiation. A separation of a blastomere at this stage causes at first a half cleavage, but the materials retain a relation to each other very similar to the normal whole relation as far as the apical-basal axis is concerned along which differentiation is assumed; the embryo, therefore, can readily adjust itself to form a whole larva, having all the necessary materials present in the proper relations.

In the fragment of the unsegmented egg this is not true. Here, according to all indications, there is a great activity in the materials of the egg. If the egg is cut at an early stage (as in the unfertilized egg) there is yet a considerable period of activity and movement of materials through which the egg must pass before the first cleavage takes place; and, therefore, on the one hand a whole cleavage results, and on the other a normal whole larva is formed. Such is not the case, however, if we take the egg for instance after maturation not long before the cleavage. The egg is nearly ready for the first cleavage, the materials are arranging themselves for an equal distribution and the proper physical tensions for such a division are present. The egg is now cut and a portion of it removed. The cleavage ensues very quickly, for the physical machinery was already starting to act when the cut was made. The different materials are not separated in a precise way, even if the cut is vertical, for the cytoplasm is semi-liquid, and the materials, especially along the injured side, come into abnormal relations with each other, which cannot be regulated as in

the earlier stages, because of the lack of the opportunity which, as stated above, is afforded the earlier ones (*c. f.* again the experiments on the frog's egg). The defects in the larvæ have a definite relation to the position of the removed part of the egg because the disturbance of the protoplasm is greatest in the region near the cut. The already differentiated materials may thus be separated in an abnormal relation to each other and become unnaturally grouped by the cell walls of the ensuing divisions. In this manner, on the one hand the abnormality of the resulting cleavages, and on the other the defects in the larvæ developed from the fragments, may be explained. The first normal cleavage, however, divides the cell into two similar parts, each of which retains a relation between its differentiated materials very much like that of the whole egg. The conclusion is therefore reached that the relations of the materials in the isolated blastomere of the two-cell stage are more normal (*i. e.*, more like those of the whole egg) than are those of fragments of the two-polar-body stage, and therefore the capacity of regulation to form a whole larva is greater in the former than in the latter. The mechanism of division which is disturbed by the cut in the unsegmented egg, and is capable of regulation if the cut is early but is disturbed if the cut is late, is also not disturbed in the isolated blastomeres of the two-cell stage, the cell division goes on as if the other blastomere were present, and a partial (one-half) cleavage results.

It therefore seems probable that while in normal development cleavage is an aid in differentiation, in development after removal of a portion of the unsegmented egg (or segmented egg) it is a distinct detriment in so far as the attainment of the normal relations of the parts is concerned. For while on the one hand it isolates materials and allows a more accurate differentiation, on the other it restricts the power of regulation of the organism.<sup>1</sup>

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<sup>1</sup>Yatsu has hinted at an explanation somewhat similar to the above. He says that the differences between the larvæ derived from fragments of the unsegmented egg and those from isolated blastomeres of the two-cell stage may be due to differences in the accuracy of the separation of the materials in the two cases.



While the larvæ developed from isolated blastomeres of the two and four-celled stage show certain organic differences from the normal whole larvæ, there is no indication in them of a specific local defect. The first trace of such a defect is reached in the eight-cell stage. Here, while larvæ developed from lateral four-cell groups containing two cells of the upper quartet and two cells of the lower show the characters of a normal larva (except for asymmetry in arrangement) larvæ from the upper quartet always possess an apical organ and lack an enteron, and those from the lower possess an enteron and lack an apical organ. There is thus a very distinct differentiation along the apical-basal (polar) axis. It is an interesting fact that this first distinctive morphogenic localization is coincident with the first inequality in cleavage, the inequality being in the same direction. Projecting backward this differentiation—that is, assuming that an apical-basal differentiation has been going on for some time before the eight-cell stage—naturally there would be no indication of it in the isolated blastomeres of the two or four-cell stages because the cleavages are vertical. Likewise there may be a similar differentiation in the unsegmented egg; for, while my results on cleavage defects cannot be analyzed as showing any specific relation to the individual kinds of egg defects, the observations of Yatsu on morphogenic defects do show such a relation.

The experiments on the eggs of *Cerebratulus marginatus*, together with the former ones on *C. lacteus*, seem therefore to indicate that at the eight-cell stage the formative materials of the egg are definitely localized in an apical-basal direction, and the experiments of Yatsu on morphogenic defects in larvæ resulting from unsegmented eggs of the later maturation stages show a similar apical-basal differentiation. That this process of apical-basal differentiation is a progressive one in the unsegmented egg is indicated by the whole character of the cleavage in fragments of unfertilized eggs, and by the progressive departure from this character up to the first cleavage, and the corresponding increase in defects of larvæ developed from the fragments, though in the latter case the continuity of the result seems to be masked by the development of whole larvæ from isolated one-half and one-fourth

blastomeres. An explanation of this has, however, been offered. The first two cleavages being perfect apical-basal ones, the isolated blastomeres cannot be expected to show other than perfect larvæ, assuming only a slight regulation overcoming lateral asymmetry, notwithstanding the partial cleavage, which after all is only quantitatively partial ( $=\frac{1}{2}$  or  $\frac{1}{4}$  of a pattern). At the same time the fragments of unsegmented eggs can never be said to contain the materials divided accurately with respect to an apical-basal axis because, in the first place, the cut is never perfectly vertical, and in the second place, the rounding in of the edges after such a cut causes a disarrangement of the materials which must result in unequal distribution at the first cleavage. The ability to regulate such an unequal distribution must of course largely depend upon its extent and character. The greater opportunity given to fragments of unfertilized eggs to regulate such differences in distribution (if any) before cleavage takes place may to some extent explain the whole character of the cleavage in such fragments without the assumption of a perfectly isotropic egg, an assumption which is contradicted by the evident polarity of the egg at this period as indicated by the eccentricity of position of the nucleus and the presence of the basal protuberance. The existence of such an apical-basal differentiation in the unsegmented egg was indeed already indicated by Professor Wilson's result on certain eggs in which the basal portion was removed by a horizontal cut and which showed the basal quartet of the resulting eight-cell stage much smaller in comparison with the upper than in the normal whole egg.

The data on localization of formative factors in the egg before cleavage and during the early segmentation stages may, therefore, be provisionally stated in the following form for *Cerebratulus*, if an intimate relation is assumed between localization of visible materials and localization of formative factors. The unfertilized egg before the beginning of maturation already shows evidences of a polarization which necessitates the assumption of a heterogeneity in material. Upon this basis, and in the same apical-basal direction, later differentiation proceeds.

During the preliminary maturation stages and after fertiliza-

tion there are profound changes in the distribution of materials in the egg, and these changes seem to be accompanied by an increased apical-basal differentiation. The first two cleavages being vertical and equal merely effect a quantitative and not a qualitative separation of materials, but the third plane of division, a horizontal one, bringing about an unequal division, separates the egg into two qualitatively different parts. That such is the case is absolutely demonstrated by my experiments on the eight-cell stage in which I obtained complete larvæ from lateral four-cell groups, larvæ with an apical organ but without an enteron from the upper quartet and larvæ with an enteron but without an apical organ from the lower quartet.

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## AN EXAMINATION OF THE PROBLEMS OF PHYSIOLOGICAL "POLARITY" AND OF ELECTRICAL POLARITY IN THE EARTHWORM.

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The so-called "polarity" exhibited in the regeneration of animals has suggested the idea to a number of writers that the phenomenon might be related to, or the outcome of differences in potential in different regions; or, in other words, of electrical polarity. The term "polarity" itself, which has been generally adopted to express a sort of stereometric relation in the regeneration of living things, suggests in certain striking ways the polar relations observable in many electrical phenomena, and invites a direct comparison between the two.

The only experiments that have been undertaken to test directly this question are the recent ones by Mathews<sup>1</sup> on certain hydroids and on the tail of *Fundulus*. The interesting results reached by Mathews, while leaving the problem, so far as the main issue is concerned, still an open one, showed the importance of further examination of the subject. Mathews avoids, it is true, making a direct comparison between physiological "polarity" and the polarity present in electrical phenomena, and speaks rather of the rate of growth of certain regions in comparison with others; but if there is in reality any fundamental relation between the phenomena in question, we should expect to find some expression of it in the polar, or, more generally, the stereometric relations of the parts.

If, for instance, the development of a head at the anterior end of a piece and of a tail at the posterior end is connected with difference of potential in the two regions, we might hope to get evi-

<sup>1</sup>Electrical Polarity in the Hydroids. A. P. Mathews. *Am. Journ. Physiology*, Vol. VIII, No. IV, Jan. 1, 1903, pp. 294-299.

dence of this by means of the galvanometer. If this relation should be found to exist, there is a further opportunity of testing the validity of the conclusion in the case of axial heteromorphosis. For this reason we selected the earthworm for our study, since in the earthworm it had been shown by one of us that there regenerates from the anterior cut surface of posterior pieces not a head, but a tail. We should expect to find under these circumstances a reversal of the potential in this region, when compared with an anterior cut surface in the more anterior regions of the worm. The following pages give the results of our examination.

#### METHODS.

Both *Lumbricus terrestris* and *Allolobophora fœtida* were used. Since the results appeared to be similar for both species, *Lumbricus*, being larger and showing on the whole greater differences of potential, was preferred when available. In all, sixty-four worms were used, the number of tests made upon any one varying from one to seventeen. Differences of potential were detected by a Rowland-d'Arsonval galvanometer, connected with a pair of non-polarizable electrodes. Since the regulation of these electrodes was found to be troublesome, and since they were liable to introduce a source of error into the readings, their manufacture and regulation had best be described. Two glass tubes about three inches long, somewhat smaller at one end, were plugged at the small end with kaolin or filter paper, moistened with normal (0.85%) salt solution. The plug extended about a half inch beyond the end of the glass tube. When made of kaolin, the end, after being used, could be easily broken off and re-formed from fresh material. When made of filter-paper, the tip, if it became contaminated from touching the worm, could be cut off. Above the plug each tube contained a saturated solution of zinc sulphate, into which projected through a cork a small amalgamated zinc electrode, connected by a wire with one of the poles of the galvanometer. Since the instrument was so sensitive that the slightest loss of equilibrium was at once registered by a deflection of the mirror, great care had to be exercised in keeping the junctions of wires and zinc electrodes dry, and in balancing the other elements



of the electrodes. As the electrodes were found to deteriorate rapidly when used, it was necessary to examine them at frequent intervals, and when the deterioration became so great as to affect seriously the value of the readings on the worm, to readjust the parts. This could be accomplished in several ways, of which sometimes one and sometimes another was most effective. A fresh zinc electrode could be substituted, the zinc electrodes could be freshly amalgamated, or they could be polished by rubbing them with sandpaper. The current could sometimes be affected by moving one zinc so that a greater or less surface was immersed in the zinc sulphate. Putting fresh zinc sulphate solution into the tube nearly always produced a distinct effect. All these means of regulation proved more or less temporary, and often, when they all failed to balance the electrodes, fresh ones had to be made. The electrodes in which filter-paper was used proved much more constant and easy to regulate than the clay ones, and if they were often renewed, it seemed safe to employ them.

A positive deflection of the galvanometer meant that the potential at the right hand electrode was higher than that at the left hand, *i. e.*, the current through the galvanometer flowed from right to left. Since in nearly all cases the left hand electrode was placed on the worm anterior to the right hand one, a positive deflection meant that the current flowed through the galvanometer from the posterior to the anterior electrode.

The electrodes were applied, sometimes both to the dorsal surface of the worm, sometimes to the ends made by transverse sections through the body, and sometimes one to the surface, and the other to a cut end. The results will be considered according to the position of the electrodes.

#### ONE ELECTRODE ON THE SURFACE AND THE OTHER ON THE CROSS-SECTION.

It will be seen from the records given in the following selected tables that however general certain results appear to be, nevertheless some individuals show irregularities. This uncertainty in the results can be in part at least accounted for by the following considerations. Secretions in different regions of the body, the

flow of blood from cut surfaces, the excretion of slime from the skin, local or general muscular contractions might all tend to affect the results. Such effects could sometimes be distinctly seen in an increase or decrease in the deflection of the galvanometer. It seemed possible that the presence or absence of food in the digestive tract might in itself or by stimulating the flow of digestive fluids influence the distribution of electric potential, but this was not observable from tests made with worms that had been starved for several days.

*Experiment 1.* *Lumbricus terrestris* starved two days.<sup>1</sup> Cut ends anterior at several levels. The left hand electrode was applied to the cross-section and the right hand electrode to the dorsal surface one-third to one-half inch behind the section. The zero point of the galvanometer was 27.1, and the effect of the electrodes varied, deflecting it between the limits 26.1 and 29.3, both of which deflections are less than those caused by the worm itself, and may therefore be disregarded. The galvanometer readings at different levels on the worm were as follows:

Cut at fourth segment. . . .	a. 45.0+ (off scale)	Surface positive
Cut at fourteenth segment. .	b. 35.5	" "
Successive sections between	c. 45.0+ (off scale)	" "
the fourteenth segment	d. 42.5	" "
and the middle of the	e. 40.0	" "
worm. . . . .	f. 43.5	" "
Successive sections posterior	g. 11.5	Cut end positive
to the middle. . . . .	h. 34.0	Surface positive
	i. 32.2	" "

The readings are all definite and represent the state of affairs in a majority of the worms examined. From these data it will be seen that when a worm is cut in two it is found that in the anterior regions of the worm the anterior cut end of the posterior piece is negative with respect to the near-lying surface. In the posterior regions of the worm where there was more variation the differences in potential were usually less, and sometimes reversed in

<sup>1</sup>Since the results seemed not to be affected by the absence of food from the digestive tract, this specimen was chosen because a more complete series of sections were made from it than from any unstarved worm.

direction. Irregularities were often observed at about the fifteenth segment, where the male reproductive organs open to the exterior and the crop-gizzard region begins. Irregularities also occurred at the girdle. In the worm used for this experiment negative deflection of the galvanometer occurred only once, immediately behind the middle of the worm, but the positive deflection was diminished at the fourteenth segment and at the other cut ends in the posterior regions of the worm.

*Experiment 2.* *Lumbricus terrestris*. Cut end posterior. The zero point of the galvanometer was 27.3, and the electrodes caused a deflection to 27.5 at the beginning and to 30.0 at the end of the experiment, which does not affect the sense of the deflection for any reading. The galvanometer record for the different levels is as follows:

Successive sections from the posterior end to the middle of the worm...	a. 28.0	Cut end positive
	b. 19.5	Surface positive
	c. 23.0	" "
	d. 23.5	" "
Section about the middle.....	e. 24.0	" "
Section anterior to middle.....	f. 25.0	" "
Section at 23rd segment.....	g. 39.0	Cut end positive
Section at 15th segment.....	h. 20.0	Surface positive
Section anterior to 15th segment....	i. 17.7	" "

The surface has a higher potential than the cut end at all but two levels, one where only a few segments are cut off from the posterior end, where the difference of potential is small; and the other at the twenty-third segment. The reversal of current at the extreme posterior end occurred in all the worms in which a full series of sections was made. The twenty-third segment is between the fifteenth segment and the girdle. In this region two other worms showed possible cases of reversal of current, though the more usual condition was that the current was reversed at the fifteenth segment or at the girdle, or at both points, but between them flowed in the same direction as in the rest of the worm. The worm used in this experiment showed the same distribution of potential that is found in a majority of individuals, except at the fifteenth segment, where one-half of the worms tested showed a reversal of



current, the other half agreeing with this experiment in showing no reversal.

From experiments 1 and 2 it may be assumed that when an earthworm is cut in two, the transverse section commonly represents a point of lower potential than the uninjured surface near to it. At the cut end chemical changes no doubt take place as a result of the fluids there set free, and of the general breaking down of tissues. These conditions might be expected to alter the electrical potential at the cut end, and presumably, where these changes are greatest, the alteration of potential will be greatest. Since, however, the transverse section is usually lower in potential than the uninjured surface near it, whether the section be at the anterior or posterior end of the piece, the difference in potential cannot bear any relation to the *kind* of regeneration that is to take place.

Having illustrated the more regular and more usual conditions of distribution of potential between surface and cross section, two illustrations of the exceptions to this condition, sometimes met with, will now be given. In the first a distribution of potential different from the average occurred throughout the whole worm. In the second, a short piece of a worm showed unusual conditions.

*Experiment 3.* *Lumbricus terrestris*, young. Cut end posterior. The zero point of the galvanometer was 27.4, and the electrodes deflected it to 26.3 at the beginning of this experiment, and to 28.9 at the beginning of the next experiment made that day. Some doubt may, therefore, be thrown on two of the readings given below—those at the girdle and at the fifteenth segment. The direction of deflection in these cases is probably correct, but the amount is small. The readings were as follows:

Posterior to middle.....	a	24.0	Surface positive
Just back of girdle.....	b	29.0	“ “
Anterior to girdle.....	c	33.5	Cut end positive
	d	34.6	“ “
At 15th segment.....	e	26.5	Surface positive
	f	32.8	Cut end positive
Anterior to 15th segment.....	g	36.0	“ “
	h	34.9	“ “
	i	35.0	“ “

Here we see the cut end with a higher potential than the surface, except at the girdle and in front of the fifteenth segment, where the current is reversed. The worm was small and immature, but as other young worms gave the same sort of readings as the majority of mature worms, the peculiar results cannot be due to immaturity. In fact, it is difficult even to guess why this worm should give such different responses from the others. Another case was also recorded in which the cross section was anterior and of higher potential than the neighboring surface for a series of seven sections.

*Experiment 4.* *Lumbricus terrestris*. Piece one inch long from near the posterior end of the worm, with a cut surface at each end of the piece. Zero point of galvanometer, 28.6; electrodes, 30.0.

Electrodes at anterior end and

middle of piece. . . . . 26.3 Anterior cut end positive

Electrodes at posterior end and

middle of piece. . . . . 27.1 Surface positive

Electrodes at both ends. . . . . 27.0 Anterior cut end positive

In this experiment the anterior electrode is positive with respect to the posterior one, whether it be on an end or on a surface, and we get a constant direction of current from before backward. The worm from which the piece was cut gave the usual results for the other readings made on it. The piece was cut out by two consecutive cuts with no appreciable time between them, so that the freshness of the cut could not, as it does in other cases given later, influence the result. One other case resembled this one, while two cases showed conditions in which the middle was positive with respect to both ends, and three cases showed conditions in which the middle was negative with respect to both ends.

It has been stated that marked changes in the distribution of potential between the surface and cross section often occur at the fifteenth segment and at the girdle region. When a worm is cut in two at the fifteenth segment the cut end has usually a higher potential than the surface either anterior or posterior to it, or when short pieces of a worm are cut with one end at the fifteenth segment, that end is positive to the other, whether it be an anterior

or a posterior end of the piece. When, however, a worm is cut in two immediately anterior or immediately posterior to the girdle, the girdle has a lower potential than either cut end. Both these regions, therefore, show a state of affairs different from that in other parts of the worm. When worms were cut in two at a series of points it was found that at the fifteenth segment there was a change in direction of the current in twelve cases out of nineteen, and at the girdle in eight cases out of fourteen, four cases in which the change was not very pronounced being included in the first series, and two in the second. The change in direction of current, though by no means uniform, is rather more likely to occur than not, and may perhaps be connected with substances secreted by the organs at these levels.

If the distribution of potential in the earthworm resembles the distribution of potential in a resting muscle, we might expect that the difference of potential between the electrodes would vary according to the position of the electrodes. A series of experiments was tried, in which one electrode was kept stationary at a transverse section, and the other moved along to different positions—usually one near, one half way between the ends, and one on the skin at the end opposite the transverse section. Great variation in the deflection of the galvanometer was always observed for these different positions, but it was by no means regular. The most common case was that the deflection was greatest when the electrodes were near one another, decreasing as they moved away, and sometimes even changing to an opposite direction when they were at opposite ends of the worm. In this series of experiments we have not only the complicating conditions already mentioned, but also the factor of resistance which would be approximately proportional to the distance between the electrodes, and if appreciable would modify the results in the way stated. The problem is one of greater complexity than that of the distribution of potential in the comparatively homogeneous tissue of the muscle, where the resistance is small. If the earthworm were homogeneous as regards electrical conductivity, and a difference of potential were set up by means of a transverse section, the point of lowest potential would be in the middle of the cut end, and of highest potential



at the opposite end of the worm. If, however, resistance varied in different parts of an unhomogeneous tissue, the difference of potential observed between two points would be a resultant between a tendency to a regular rise of potential and an irregular distribution of resistance, and the recorded distribution of potential would, therefore, be irregular. In point of fact, however, as mentioned above, other causes of irregularity may be added to those due to resistance, and many arrangements of potential were observed in the seventeen cases tested.

#### ELECTRODES APPLIED TO TWO POINTS ON THE SURFACE.

*Experiment 5.* *Lumbricus terrestris*. The zero point of the galvanometer was 28.6, and the electrodes deflected it to 27.9 or 27.8, in an opposite direction from deflections caused by the worm. This experiment was made in order to see what the electrical conditions are on the surface of the worm. Three readings were taken, as follows:

- a. Earthworm cut in two at the middle.  
 Posterior piece. One electrode on the surface at the section and the other on the surface, posterior to the section, but near it. . . . . 30.3    Posterior positive
- b. Same worm. Short piece cut from posterior half of animal.  
 One electrode on the surface at the anterior section, and one on the surface posterior but near. . . . . 33.9    "    "  
 One electrode on the surface at the anterior section, and one on the surface at the posterior section. 35.0    "    "

The direction of current was the same as would be expected if one electrode were applied directly to the cut end, and the other to the uninjured surface near the end. On the assumption that at any level the conditions of the surface fairly represent those in the interior of the worm, by testing the distribution of potential at the surface of an uninjured specimen it may be possible to get some

idea of the distribution within the worm. This was done in the following experiment.

*Experiment 6.* *Lumbricus terrestris*. Both electrodes applied to the surface. The zero point of the galvanometer was 26.9, and the electrodes varied from 26.7 to 22.0 after the third reading, when they were regulated to 24.3, and at the end registered only 26.0. The readings of the galvanometer were as follows:

One electrode at anterior end, the other		
near.....	32.0	Head end negative
One electrode at anterior end, the other		
at middle .....	30.0, then up, off scale.	Head end negative
One electrode at anterior end, the other		
at posterior end .....	16.0	" " positive
One electrode at posterior end, the		
other at middle.....	28.0	Tail end positive
One electrode at posterior end, the		
other near.....	18.0 } 22.5 }	" " negative

It is difficult to explain these data so that they are consistent. There is no one point that has a high potential relative to all others, though the deflections are sufficiently strong to indicate that they are not due to variability in the electrodes themselves. The variation may perhaps be due partly to local muscular contraction and partly to the excretion of slime at points on the surface, for in *Allobophora*, where the body cavity fluid extruded through the dorsal pores is yellow and noticeable, its excretion was observed to have a great effect upon the deflection of the galvanometer.

Different worms, too, show the greatest differences as to their reactions when electrodes are touched to different parts of their surface. In general, the two ends tend to have a lower potential than other parts of the surface, and the middle tends to have a higher potential with respect to points on either side of it. At the girdle and at the fifteenth segment, however, the results are more definite, as is shown in the two following experiments.

*Experiment 7.* *Lumbricus terrestris*. Zero point of galvanometer, 28.6; deflection caused by electrodes to 30.5.

One electrode at girdle, the other anterior to it, and near.....	20.5	Girdle negative
One electrode at girdle, the other posterior to it, and near.....	39.0	" " "

*Experiment 8.* *Lumbricus terrestris*. Zero point of galvanometer, 28.6; deflection caused by electrodes to 30.0, at end of experiment.

One electrode at 15th segment, the other anterior to it and near..	29.8	15th segment negative, probably
One electrode at 15th segment, the other posterior to it and near....	31.5	" " "

The girdle is definitely of a lower potential than the surface near it, anterior or posterior, and this was found to be the case for four worms tested. At the fifteenth segment the difference was not so great, and though this region was negative with respect to a surface posterior to it, with respect to one anterior it was only very slightly, or, perhaps, not at all so. In another worm the fifteenth segment was evidently positive with respect to a surface anterior to it.

#### ELECTRODES APPLIED AT TWO TRANSVERSE SECTIONS.

*Experiment 9.* *Lumbricus terrestris*. Pieces cut out from worm. The zero point of the galvanometer was 27.3. The electrodes deflected it to varying amounts. In two cases, namely the fourth and the seventh readings in the table, where the electrodes deflected the galvanometer to 26.1 and to 29.5, respectively, these deflections come near the readings given by the worm. In the other cases it is not necessary to take deflection caused by the electrodes into account, since they would not affect the direction of the reading. The data are as follows:



(a)	Anterior half of worm, both ends cut (long piece) . . . . .	16.6	Anterior positive
(b)	Short piece from anterior part of an- terior half of worm, the posterior end cut somewhat later than the anterior end . . . . .	22.3	" "
(c)	Same. Anterior end freshly cut. . . .	32.5	Posterior positive
(d)	Short piece from middle part of an- terior half of worm, posterior end more freshly cut. . . . .	27.1 27.0 27.9	" "
(e)	Same piece, anterior end freshly cut.	36.2	" "
(f)	Short piece from anterior part of pos- terior half of worm. . . . .	33.0	" "
(g)	Short piece from middle of posterior half of worm. . . . .	{ 28.5 24.0	Anterior end positive probably
(h)	Anterior half of (g), posterior end freshly cut . . . . .	22.0	Anterior positive
(i)	Posterior half of (g), anterior end freshly cut. . . . .	34.0	Posterior positive

In this worm, when the two ends were cut at approximately the same time, which happened in (a), (f) and (g), the piece from the anterior half had its anterior end positive, and the two pieces from the posterior half had their posterior ends positive. In the majority of worms tested, when the two ends of a piece were cut at the same time, the anterior end was positive, regardless of the position of the piece on the worm.<sup>1</sup> If, however, the two ends were cut at different times, which in this worm occurred in six pieces, the end which had been cut most recently generally had a lower potential than the other. Since the testing with the electrodes on a transverse section and an uninjured surface near that section the end was usually found to be at a lower potential than the surface, the fall of potential was supposed to be due to

<sup>1</sup>When, however, the pieces were long (one-half the worm or more), in a majority of cases the posterior end was positive with respect to the anterior.

changes accompanied by the escape of body fluids at the cut end. Since, when the electrodes are on two transverse sections, the one that is more recently cut is of a lower potential than the other, it would appear that the causes that determine the fall of potential are such as decrease in the course of a short time. The fluids that escape from the cut end dry rather rapidly, whereas the tissue cells, breaking down, are not built up for several days. The first fall of potential, then, is probably largely due to escape of blood or other fluids from the section, while the slighter permanent effect may be due to the breaking down of tissue cells. When there was but a short time between the two cuts the lower potential did not always occur at the fresher one, which may have been partly because fresh fluids were still coming from the earlier section. In the anterior half of the worm, also, there was great irregularity, which may be partly due to different digestive fluids at different regions of the digestive tract producing varying electrical activities.

The differences of potential between two transverse sections are probably a resultant of the factors that cause difference of potential between a section and a surface. If a piece be cut out from an earthworm by two transverse sections, there will be a difference of potential between each end and the surface between the ends. If the differences of potential between each end and the surface are equal and opposite they will balance one another, and there will be no difference of potential when electrodes are applied to the two ends. If, however, the differences of potential between the two ends and the surface are unequal, their resultant will determine a difference of potential between the two ends. This is illustrated by experiment 10.

*Experiment 10.* *Lumbricus terrestris.* Short piece. The zero point of the galvanometer was 28.6, and the electrodes registered slightly above this at the beginning of the experiment. The readings were as follows:

- |   |      |
|---|------|
| (a) One electrode applied to anterior end, the other to the middle of the piece.....  | 25.0 |
| (b) One electrode applied to posterior end, the other to the middle of the piece..... | 30.8 |

- (c) One electrode applied to one end, the other to the other end of the piece..... 28.0

If we disregard the deflections caused by the electrodes, the following are the departures from the normal: (a.) = - 3.6, (b.) = +2.2, (c) = - 0.6. Theoretically, if (c.) were the resultant of (a.) and (b.), it would equal - 1.4, but considering how variable the conditions were always found to be, - 0.6 presents a fairly close agreement. In all other cases but one, where similar tests were made, the results agreed in like manner with the theory.

#### REGENERATING WORMS.

In addition to the readings made from worms that had been freshly cut in two, a series of readings were made on worms in which the process of regeneration had proceeded for a number of days. The regenerating worms were divided into four groups: (1) those in which a few anterior segments had been cut off and regeneration was taking place at the anterior end of the long piece; (2) those in which the worm had been cut in two in the middle and regeneration was taking place at the posterior end of the anterior piece; (3) those in which the worm had been cut in two in the middle and regeneration was taking place at the anterior end of the posterior piece; (4) those in which the worm had been cut in two at the fifteenth segment and regeneration was taking place at the anterior end of the posterior piece. They were allowed to regenerate from twenty-five to thirty-two days, and in the course of that time five or six tests were made on most of them at intervals of a few days. One electrode was applied to the regenerating tip, and the other to the old surface a short distance from the tip. In all one hundred and fourteen readings were recorded, the results of which may be summarized as follows:

Group (1) .....	31	cases, end positive; 9 cases, end negative
Group (2) .....	20	" " " 18 " " "
Group (3) .....	15	" " " 11 " " "
Group (4) .....	8	" " " 2 " " "
Total .....	74	" " " 40 " " "



If we regard only the readings made when regeneration had proceeded more than twenty-one days, in thirty-six cases the end was positive with respect to the surface, and in eleven cases it was negative, a much more definite result than when all cases are considered.

The conditions in a regenerating tip do not, therefore, agree with those at a freshly cut end, for the current flows in an opposite direction. To be sure, the processes occurring during regeneration are not the same as those occurring during the breaking down of tissues, and the latter may be the predominant ones immediately after a cut is made. The subject, however, needs further investigation before the causes for this reversal can be more than surmised.

#### ELECTRICAL POLARITY AND RATE OF REGENERATION.

If we look for a relation between electrical polarity in the worm and rate of regeneration, as Mathews has suggested, we find it as difficult to demonstrate as the difference between electrical and physiological polarity. If the average deflection of the galvanometer was greater at certain levels where regeneration is known to be rapid than at other levels where it is slow, the connection would be established. For instance, the regeneration of a tail at the posterior end of a worm when only a few posterior segments are cut off, is exceedingly rapid, whereas the regeneration of a heteromorphic tail at the middle of a worm is very slow. The average deflection of the galvanometer in the former case for three readings is, however, 3.4, with the cut end positive instead of negative in every case. In the latter case the average deflection for seven readings is 3.6 (with the end negative), with no extreme readings to bring it up. The region in the middle of the worm, where a tail is to regenerate from the posterior end of the anterior piece, gives an average deflection of 4.7 for seven readings from different worms. When five or six segments are cut from the anterior end of the worm the average deflection for seven cases was 4.6 at the anterior end of the long piece. At the posterior end of the short piece, regeneration would be very slow, and at this end

only two readings were made, one giving a very slight result, the other deflecting the galvanometer to 5.2.

If we attack this subject by another method, namely, by making a direct comparison between two freshly-cut ends on one worm, the results are equally indefinite. When five or six segments were cut from each end of the worms, of a series of fifteen readings on different worms made with one electrode at the anterior-cut end and the other at the posterior-cut end, the posterior end was positive in eleven and the anterior end positive in four. When the worm was cut through the middle and at the posterior end, the posterior end was positive in three cases, and the middle in one case. When the cuts were made through the middle and anterior end, in five cases the middle was positive, and in two the anterior end positive. From these considerations it would therefore appear that no invariable connection between rate of regeneration and electrical polarity exists in the earthworm, at least as measured on a freshly cut surface.

From the foregoing experiments we conclude:

(1) That a freshly cut end of an earthworm is generally negative with respect to a near-lying uninjured surface.

(2) That the freshness of the cut surface has an important influence in determining the amount of difference of potential.

(3) That the result is often complicated by the presence of secretions or exudations on the surface, or by the presence of certain organs at the cut end, or by the contractions of the worm, etc.

(4) That in the region of the girdle and also in the region of the fifteenth segment (near which the crop and gizzard lie), the results are often different from those elsewhere.

(5) That there is no apparent relation between the differences in potential at freshly cut surfaces and the kind of regeneration (head or tail) that occurs.

(6) That cut surfaces from which heteromorphic growth would take place show the same sort of differences in potential as those from which orthomorphic regeneration occurs.

(7) That the differences in potential present when a cut surface is exposed can probably be accounted for by the chemical changes taking place at the surface; and these need have, and do

not appear to have, any relation to the kind of regeneration that takes place.

(8) That when, on the other hand, the cut surface is allowed to heal, and when later a new structure has begun to appear, the differences in potential between the new and the old parts (*as measured on the surface only*) are not such as can be made to account for the difference in the kind of part (head or tail) that is regenerating. Here also many complications enter into the result and make it difficult to draw satisfactory conclusions.

(9) No definite relation was found between the rate of growth and the fall of potential between an uninjured surface and a cut end.





## THE REGENERATION OF A HETEROMORPHIC TAIL IN ALLOLOBOPHORA FOETIDA.

ABIGAIL CAMP DIMON.

In a paper by Professor Morgan<sup>1</sup> an account was given of anterior regeneration from three different levels in earthworms. The results seemed to show that the internal factor determining the formation of a heteromorphic tail might be the presence of the stomach-intestine at the regenerating surface, and at Professor Morgan's suggestion and under his direction, the following experiments were undertaken. An attempt was made to test this view by means of more exactly localized sections made near the level of the beginning of the stomach-intestine.

In *Allolobophora foetida*, the species used, the œsophagus extends to the fifteenth segment, the crop lies in the fifteenth and sixteenth, the gizzard in the seventeenth and eighteenth, and at the nineteenth begins the stomach-intestine, which extends posteriorly through the rest of the worm. The external openings of the vasa deferentia on the fifteenth segment served as convenient landmarks for determining the level of the section. The worm was cut in two, the short anterior piece dropped into alcohol, and its number of segments counted so that the exact level of the cut could be recorded. The posterior piece was then left to regenerate from forty-eight to one hundred and twenty days, when it was killed, and sections made for study. In some cases a new head, and other cases a new tail regenerated from the anterior end of the posterior piece. In the regenerating head the new stomodæum usually did not open into the old digestive tract, which closed anteriorly, and no definite pharynx formed. A dorsal brain, connected with the ventral nerve cord was usually present. Since these conditions represented the most usual form of head regenera-

<sup>1</sup>Experimental Studies of the Internal Factors of Regeneration in the Earthworm. Arch. für Entwicklungsmech. der Organ. Bd. XIV. pp. 562-591.

tion at the levels of these experiments, the cases in which they exist are classified in the table as a separate group under Head A. Cases where the brain lies anterior and even ventral to the level of the digestive tract; where the nerve cord ends without forming a brain; or where there is no mouth invagination, though the brain is well developed, are classified as Head B. The heads of group B look less like a normal head than those of group A, and yet are very evidently to be classified as heads rather than as tails. The distinctive features indicating a heteromorphic tail are the formation of a number of segments, the opening of the digestive tract to the exterior through a new anus, and the ending of the nerve cord ventrally, without a brain. Tails possessing these features are put in group A, while those in which any of these features are absent, are put in group B.<sup>1</sup>

In all, one hundred and seventeen worms were examined, with the results given in the table. The number of the segment given at the head of each column locates the level at which the worm was cut in two, and both the actual number of worms and the percentages are given under each class.

	12th- 13th Segment		Between 14th Segment and 15th Segment		Between 15th Segment and 16th Segment		Between 16th Segment and 17th Segment		Between 17th Segment and 18th Segment		Between 18th Segment and 19th Segment		Back of 1th Segment	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Head, A...	2	100	7	70	11	46	7	59	6	46	6	18	...	...
Head, B...	...	...	3	30	11	46	4	33	6	46	20	61	4	17
Tail, B...	...	...	...	...	...	...	...	...	...	...	1	3	5	22
Tail, A...	...	...	...	...	...	...	...	...	...	...	1	3	7	30.5
Uncertain	...	...	...	...	2	8	1	8	1	8	5	15	7	30.5
Total	2	100	10	100	24	100	12	100	13	100	33	100	23	100

<sup>1</sup>In only twenty-four cases out of one hundred and seventeen did the old digestive tract open to the exterior through the new mouth or anus. This occurred ten times in head regeneration, ten times in heteromorphic tail regeneration, and four times in cases classified as uncertain. Seven of the twenty-four cases occurred when the worm was cut in two in front of the sixteenth segment, and the other seventeen when it was cut behind the eighteenth segment.



Since there were but few worms cut further back than the eighteenth segment, and since the stomach-intestine begins at this level, all the observations made on worms cut posteriorly to the eighteenth segment were brought into one class. It is worth noting, however, that of the four heads regenerating at these levels, three formed from worms cut at the nineteenth segment, while the fourth formed from a worm in which the exact level of the cut was not noted. The percentages in the different classes, though based on a small number of cases, yet bring out clearly one or two points. When a worm was cut in two in front of the stomach-intestine, in no case was a heteromorphic tail formed. The percentage of cases in which a head was formed grows less as the section is made further back on the worm, the fall of percentage being very great immediately behind the gizzard. This tends to support the hypothesis that the formation of a heteromorphic tail is favored by the presence of the stomach-intestine near the cut end, though when the section is not more than one segment back of the gizzard a head is sometimes formed.

Though the preceding experiments seem to show that the development of a heteromorphic tail is connected with internal structures in the worm, they leave untouched the question of the kind of regeneration that takes place from posterior ends of anterior pieces cut anterior to the stomach-intestine. This point should be determined, and I hope in the future to undertake a set of experiments in which the posterior regeneration from anterior pieces will be observed.



## RESTORATIVE REGENERATION IN NATURE OF THE STARFISH *LINCKIA DIPLAX* (MÜLLER AND TROSCHEL).

BY VERNON L. KELLOGG, STANFORD UNIVERSITY, CALIF.

On the surface of the coral reefs guarding the harbor of Apia (Samoa) the five-rayed starfish, *Linckia pacifica*, with its long, slender, smooth, sky-blue arms, is the most conspicuous and abundant echinoderm in a place where echinoderms abound. Associated with it, and similarly blue and conspicuous, although smaller,

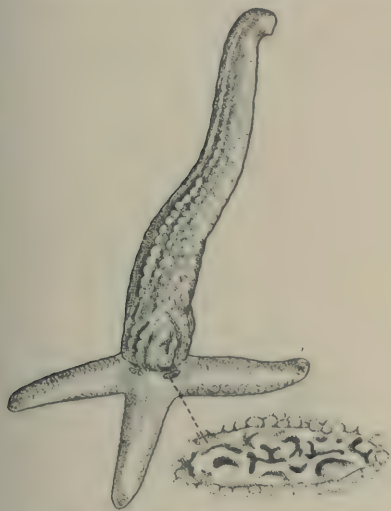


FIG. 1. *Linckia diplax*, regenerating from a single arm; note these new arms and new disc with madreporites.



FIG. 2. *Linckia diplax*, regenerating from a single arm; note four new arms and new disc with madreporites.

is the species *L. diplax*. Both for number of species and wealth of individuals, the Apia reefs are distinguished by their starfish, sea-urchin and holothurian fauna. In collecting on these reefs during several weeks in the summer of 1902, as a member of



the U. S. Bureau of Fisheries' Samoan Explorations party, my attention was particularly attracted by the many examples of starfishes with regenerating arms, and I gave some special care to picking up such specimens. From this material the figures here presented have been drawn and in themselves tell how effectively this capacity for restorative regeneration obtains in this species.

Morgan calls attention in his "Regeneration" (1901, p. 102 and elsewhere) to the assertions of some authors that starfishes



FIG. 3. *Linckia diplax*, regenerating from a single arm.



FIG. 4. (a) *Linckia diplax*, a single arm broken at both ends regenerating. (b) Aspect of proximal end of arm.



FIG. 5. *Linckia pacifica*, regenerating from a single arm, broken off obliquely from the original disc; note four new arms and disc, the outer arms larger than the two inner ones.

can regenerate a new disc and other arms from an arm torn off without any part of the disc attached, and to the denials by other authors that such radical restoration can take place. In the case of *Linckia diplax* there seems to be no doubt of the capacity of an arm torn off at some distance from the disc to regenerate a complete new animal from its proximal surface. The possibility that these arm pieces were thrown off by autotomy instead of being torn off by enemies may be noted, but such a condition makes the

regenerative phenomena none the less interesting. I have seen no example of the regeneration of several new arms (or a new disc and arms) from the distal end of a mutilated arm, as observed by the Sarasins in *Linckia multifera* (Ergeb. Naturforsch. auf Ceylon, 1884-85, I, Wiesbaden, 1888). In all cases of regeneration from the distal end of an arm noted among the Apia reef starfishes, simply a continuation, in straight line, of the tapering tip occurred. Among the figures will be noted the illustrations of three specimens in which the regenerating arm has had its distal end

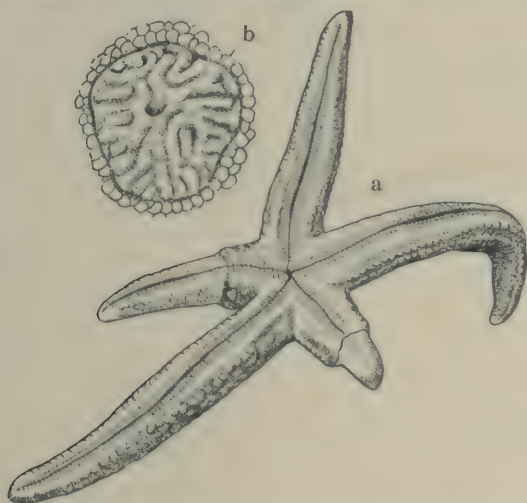


FIG. 6. *Linckia diplax*, (a) a specimen regenerating parts of two arms: (b) the aspect of a normal madreporite (compare with the regenerated madreporite shown in figures 1 and 2).

torn off (or thrown off) as well as having been itself broken off from its basal extremity, and thus freed from the rest of the body to which it originally belonged. In all of these cases of mere segments of a single arm regeneration is proceeding at both mutilated ends.

In Figures 1 and 2 a new mouth and both<sup>1</sup> madreporites are in the regenerated part. In Figure 3 a new mouth has been already regenerated, but no madreporite as yet. In Figure 4 is shown an

<sup>1</sup>*Linckia diplax* is characterized by the possession of two madreporites.

arm torn off at some distance from the disc, just beginning to regenerate. The cut end has "calloused" over, apparently by the inbending of the edges of the body wall, but in the center is left a small opening (serving as mouth?). No protuberance indicating new disc or arms has yet appeared. The arm segment, which is regenerating at both ends, shown in Figure 5, is of another species of *Linckia*, probably *pacifica*, and had an obliquely cut surface at the proximal end, and the two outer arms of the four regenerating ones, that is, those nearest the parent arm, are about twice as well developed (as far as size goes) as the other two. No madreporite is yet developed on the new discal portion. The specimen illustrated in Figure 1 is regenerating but three new arms instead of the normally missing four. In all the specimens illustrated by Figures 1, 2, 3, 4 and 5 the arms were undoubtedly broken off without any part of the disc attached.

## NOTES ON INSECT BIONOMICS.

BY V. L. KELLOGG AND R. G. BELL, STANFORD UNIVERSITY,  
CALIFORNIA.

In connection with the experimental breeding and rearing under controlled conditions of food supply of many lots of silkworms (*Bombyx mori*) during the last three years, the writers have made certain observations and experiments incidental to the main object of the investigation, the results of some of which may be here briefly abstracted.

### *Food Conditions in Relation to Sex Differentiation.*

It has been assumed by some authors that poor nutrition of developing organisms is an extrinsic influence tending to determine the sex of the organism to be male and good nutrition an influence tending to produce females. The most important part of the assumption is the idea that sex is subject to control by the environment of the organism—that sex is not inherently predetermined in the germ.

From the notes of the writers recording the results of an experimental rearing of numerous lots of silkworms on reduced rations in 1901, 1902 and 1903, the following data are extracted touching the problem of the relation of nutrition to sex differentiation. From an inspection of these data it will be noted that a test is included of the possible influence of poor nutrition of the parents (and grandparents) in determining the sex character (if predetermined) of the germ cells, as well as of the possible immediate influence of nutrition in determining the sex of developing individuals. It will be noted also that we have had in mind the justly made criticism of most observations on the food and sex problem, namely, that no attention is paid in records of an apparent overproduction of males following poor nutrition, to the deaths which ensue before the count is made, and that, as the females (it being assumed) actually require more food to complete their de-



velopment, the preponderance of males is due to the untimely death of the females.

A series of lots of ten individuals each were reared in 1903, with the specific intention of testing the assumed influence of nutrition on sex determination. These lots included: (a) a lot underfed during the whole of larval existence; (b) a lot underfed during the second to fifth intermoult periods, inclusive; (c) a lot underfed during the third to fifth intermoult periods; (d) a lot underfed during the fourth and fifth intermoult periods; (e) a lot underfed during the first intermoult period only; (f) a lot underfed during the second intermoult period only; (g) a lot underfed during the third intermoult period only; (h) a lot underfed during the fourth intermoult period only; (i) a lot underfed during the fifth intermoult period only. From the rearing of such lots it was hoped to determine what influence reduced rations might have on the determination of sex, and also, if any, at what time in the larval life the influence was most potent. A consideration of the records of the rearing of these lots at the end of the season compels us to say: that the lots were much too small to afford trustworthy generalizations; that dissections of the larvæ at various ages reveal an unmistakable differentiation in sex (indicated by gross differences in the reproductive glands) at a time as early at least as the beginning of the third intermoult period, so that experimental lots *c*, *d*, *g*, *h* and *i* were distinctly superfluous; but finally, that it may be affirmed from the meager data afforded by experimental lots *a*, *b*, *e* and *f*, that the reduction of the food supply (this reduction brought as near as possible to a living minimum) did not produce any unmistakable results in the way of an overproduction of males.

Data of more interest are those derived from an inspection of the records of the experimental rearing of various larger lots of silkworms in 1901, 1902 and 1903. In 1901 the records for five lots of twenty larvæ each may be referred to:

Lot 1—Fed optimum food; no deaths before emergence of moths; produced 8 males, 12 females.

Lot 2—Fed optimum food; 2 deaths before maturity; produced 7 males, 11 females.

Lot 3—Fed one-half (approx.) of optimum of food; 4 deaths before maturity; produced 10 males, 6 females.

Lot 4—Fed living minimum of food; 3 deaths before maturity; produced 10 males, 7 females.

Lot 5—Fed living minimum of food; 6 deaths; produced 9 males, 5 females.

Four lots of twenty larvæ each reared in 1902 may be referred to.<sup>1</sup> These larvæ were the offspring of parents of the variously fed 1901 lots, and the character of the food supply of the parents is indicated as well as that of the larvæ themselves.

Lot 1—Fed optimum; born of optimum food parents; no deaths before maturity; produced 12 males, 9 females (21 individuals in this lot by mistake).

Lot 2—Fed minimum food; born of optimum food parents; 7 deaths before maturity; produced 8 males, 5 females.

Lot 3—Fed optimum food; born of minimum food parents; 11 deaths before maturity; produced 6 males, 3 females.

Lot 4—Fed minimum food; born of minimum food parents; 3 deaths before maturity; produced 11 males; 6 females.

The records of eight lots of twenty-five larvæ each reared in 1903 may be referred to. The food supply condition of the parents and grandparents, as well as of the 1903 progeny, are given. ("O" indicates optimum food, "M" indicates minimum food).

Lots	Fed	Parents	Grand-parents	Deaths before maturity	Males produced	Females produced
1.....	O	O	O	2	13	10
2.....	M	O	O	2	14	9
3.....	O	M	O	3	8	14
4.....	M	M	O	6	8	11
5.....	O	O	M	0	15	10
6.....	M	O	M	0	11	14
7.....	O	M	M	20	2	3
8.....	M	M	M	21	2	2

<sup>1</sup>Because of backward season all 1902 larvæ were fed for their first 20 days (=about one-third of whole larval life) on food of a poor quality, namely, lettuce and mulberry buds.

The writers present these figures, actual data, for what they may be worth. Like the data of the smaller lots previously referred to, they at least show that individuals living through their whole post-embryonic life on the smallest food supply capable of sustaining life, a supply varying from  $\frac{1}{4}$  to  $\frac{1}{8}$  of the supply normally used by individuals of the species, do not necessarily become males. Whether the figures indicate an appreciable influence of this nutrition on the determination of sex can be determined by the readers as well as by the writers. In the rearing season (March to June) of this year (1904), the writers purpose devoting much larger lots of individuals to the continuation of the experiment.

### *Forced Pupation.*

Experiments were made to determine how early in larval life the food supply could be cut off without stopping the metamorphosis (development) of the silkworm, whether such forced abbreviation of the food-taking period results in any unusual structural or physiological modification in the stages which follow the withdrawal of food, and whether the metamorphosis (in particular, pupation) is hastened when food is withdrawn in late larval life, an adaptation often assumed to be possessed by Lepidoptera. Such an adaptation would obviously be of real advantage, as it might often save individuals from death due to a sudden disappearance of the food supply, or to a sudden accidental incapacity to gain access to the food supply.

The silkworm spends normally about sixty days in the larval (feeding) stage, divided into five actively feeding intermoulting periods of about ten days each, by four brief two-day moulting periods, during which no food is taken. On the eleventh or twelfth day (from 270 to 300 hours) after the fourth moult, the larva "spins up" and pupates.

Twenty healthy silkworms were selected at random from a large lot (several hundred) which had been reared in one tray, all the individuals, of course, under the same condition of food supply, temperature, humidity, light, etc. Of the twenty, one was fed as long as it would take food; the other nineteen were de-

prived of food variously from the time of the fourth moult, from one day after the fourth moult, from two days after, from three days after, and so on until individuals were obtained representing a withdrawal of food supply for a period of but a day before the normal time of giving up eating to begin spinning, through periods of two days before, three days before, four, five and so on to twelve days before, the twelve-day period being the whole of the feeding period normally lasting from the fourth moulting up to spinning time. The following table displays the conditions and results of the experiment:



No. of individual.	Date of completion of 4th moult.	Date of withdrawal of food.	Date of commencement of spinning.	Date of completion of cocoon.	Death of larva if it occurred.	Wt. of cocooned pupa 4 days after larva began spinning.	Date of emergence of adult.	Wt. of adult.	Sex of adult.
1 (normal)	May 13, 11 a. m.	*294 hrs. after.	May 25, p. m.	May 27, p. m.	.....	.925 g.	June 8	.464 g.	♂ ♀
2	May 14, 11 a. m.	288 "	May 24, .....	May 26, p. m.	.....	1.085 g.	June 7	.505 g.	♂
3	May 13, 11 a. m.	248 "	May 24, p. m.	May 27, .....	.....	.980 g.	Did not issue	.....	♂
4	May 13, 11 a. m.	240 "	May 25, .....	May 27, .....	.....	.845 g.	June 8	.212 g.	♂
5	May 13, 11 a. m.	240 "	May 24, p. m.	May 25, p. m.	.....	1.003 g.	June 8	.429 g.	♂
6	May 13, 11 a. m.	210 "	May 24, a. m.	May 26, a. m.	.....	.600 g.	June 8	.177 g.	♂
7	May 12, 5 p. m.	192 "	May 25, a. m.	May 27, p. m.	.....	.809 g.	June 8	.426 g.	♂
8	May 12, 5 p. m.	192 "	May 24, .....	May 26, .....	.....	.625 g.	June 7	.165 g.	♂
9	May 29, a. m.	168 "	June 5, p. m.	June 6, p. m.	.....	1.016 g.	June 22	.554 g.	♂
10	May 29, a. m.	144 "	June 5, .....	June 6, .....	.....	.814 g.	June 23	.240 g.	♂
11	May 12, 5 p. m.	120 "	May 26, .....	See note 1	.....	.....	Did not issue.	.....	♂
12	May 12, 11 a. m.	96 "	See note 2	.....	May 29	.....	.....	.....	♂
13	May 12, 11 a. m.	96 "	See note 3	.....	May 22	.....	.....	.....	♂
14	May 29, .....	72 "	See note 4	.....	June 8	.....	.....	.....	♂
15	May 11, 4 p. m.	72 "	No spinning.	.....	May 20	.....	.....	.....	♂
16	May 29, a. m.	48 "	See note 5	.....	June 8	.....	.....	.....	♂
17	May 11, 4 p. m.	48 "	No spinning.	.....	May 19	.....	.....	.....	♂
18	May 12, 11 a. m.	24 "	No spinning.	.....	May 19	.....	.....	.....	♂
19	May 11, .....	May 11	No spinning.	.....	May 17	.....	.....	.....	♂

\*This larva, normally reared, voluntarily ceased feeding 24 hrs after the 4th moult; in the other 18 cases, the food was withdrawn before the larva had voluntarily ceased feeding at the time after the 4th moult indicated by the phrase "X hours after."

Note 1—Bottom of bottle in which larva was confined was lined with threads May 26; threads extended up one side May 27; threads swung across bottle from side to side May 28, and larva actively spinning very damp threads; on May 29 larva was spinning a closely woven circular carpet on bottom of bottle, and on May 30 larva pupated on this carpet (no cocoon).

Note 2—Bottom of bottle lined with threads May 26; larva still slowly spinning random threads May 28.

Note 3—Bottom of bottle lined with silk coating May 18.

Note 4—Larva lined bottom of bottle with stray threads before dying.

Note 5—Slight progress in the spinning by June 6, P. M.; bottom of bottle lined with threads.

From these results it may be said that silkworms may be cut off from a food supply nearly seven days before the normal limit of their feeding time and yet complete their development (spin, pupate and emerge as imago). These seven days represent a little more than half of the last intermoulting actively feeding period, or about one-ninth of the whole larval (feeding) life. The deprivation of food for from one to four days seems neither to hasten the metamorphosis nor to modify it appreciably, nor to result in the production of a moth of lessened size or lessened fertility. The larvæ deprived of food not more than four days before normal close of feeding time do not immediately spin and pupate, but wait restlessly for the normal time of pupation (approximately twelve days after the fourth moulting), and then normally spin and pupate. If deprived of food for more than four days and less than seven, the larvæ shorten their last intermoulting stage to about seven days, forming, however, a normal cocoon and transforming into a normal moth. If the larvæ are deprived of food eight days or more before their normal spinning-up time, they invariably die without forming a cocoon, and in only one case was pupation accomplished. A beginning at spinning (see notes) is made by larvæ fed for more than two days after the fourth moulting, but no spinning at all is done by larvæ deprived of food from the day of fourth moulting or from the first or second day thereafter.

The twentieth larva of the lot was to be deprived of food 216 hours after the fourth moult, but it began spinning up in 200 hours (eight days) after, and pupated on the following day. Here is a normal variation of four days out of the usual twelve of the last feeding stage, just about as much shortening as the extreme that could be induced by actual deprivation of food.

### *Loss of Weight During Pupal Life.*

A belief among commercial breeders of silkworms that there is a loss in weight of the cocoons (silk) accompanying pupal life is indicated by their recognized wish to make an early sale of the cocoon product. This loss is generally attributed to "evaporation from the cocoon." The question arose as to whether the loss in weight of the pupa-containing cocoon might be not a loss in weight of silk but an accompaniment of developmental changes in the pupa, a process in which stores of nourishment (in the larval body) are being converted into moth with chemical changes which might occasion some loss in weight. Therefore in four individuals the cocoon and pupa were weighed separately once each day from the time of pupation to time of emergence of the moth, while at the same time the daily weights of the naked chrysalids of three other lepidopterous species were determined to see if a loss of weight accompanied pupal aging in them as well as in the silkworm moth. The following table shows plainly the results of these observations:

	May 18 (2 p. m.)	May 19 (4 p. m.)	May 20 (4 p. m.)	May 21 (3 p. m.)	May 22 (6 p. m.)	May 24 (6 p. m.)	May 25 (4 p. m.)	May 26 (3 p. m.)	May 27 (4 p. m.)	May 28 (4 p. m.)
Silkworm No 1.										
Pupa .....	1.100 g.	1.094	1.093	1.085	1.076	1.064	1.060	1.049	1.041	1.027
Cocoon.....	.136 g.	.129	.125	.125	.125	.125	.125	.125	.125	.125
Silkworm No. 2.										
Pupa .....	1.153 g.	1.140	1.123	*1.130	1.123	1.101	1.098	1.090	1.074	1.060
Cocoon.....	.132	.131	?	*.133	.131	.131	.131	.131	.131	.131
Silkworm No. 3.										
Pupa .....										
Cocoon.....						.945	.942	.935	.928	.920
						.135	.130	.130	.130	.130
Silkworm No. 4.										
Pupa .....						1.073	1.066	1.055	1.048	1.040
Cocoon.....						.134	.132	.132	.132	.132
Checker spot butterfly. ( <i>Melitaea</i> sp.) Pupa .....	.394	.391	.388	.370	.363	Butterfly issued.				
Tent caterpillar moth. ( <i>Chistiocampa</i> sp.) Pupa ..	.365	.351	.338	*.345	*.344	.327	.318	.313	.308	.297
Mourning-cloak butterfly. ( <i>Euvanessa antiopa</i> ) Pupa..			.925	.854	.788	.650	.546	.462	.390	.355

\*Pupa was lifted with fingers at time of weighing instead of with forceps; the little moisture adhering would produce the difference in weight.



	May 29 (4 p. m.)	May 30 (4 p. m.)	May 31 (5 p. m.)	June 1 (3 p. m.)	June 2 (7 p. m.)	June 3 (3 p. m.)	June 4 (3 p. m.)	June 5 (6 p. m.)	June 6	June 7
Silkworm No. 1. Pupa .....	1.007	.984	.964	.950	Moth issued.					
Cocoon .....	.126	.125	.125	.125						
Silkworm No. 2. Pupa .....	1.042	1.020	Moth issued.							
Cocoon .....	.131	.131								
Silkworm No. 3. Pupa .....	.912	.901	.890	.880	.867	.856	.831	.803	Moth issued.	
Cocoon .....	.130	.130	.130	.130	.130	.129	.129	.127		
Silkworm No. 4. Pupa .....	1.031	1.019	1.007	.997	.975	.961	.935	.910	.880	Moth issued.
Cocoon .....	.132	.132	.132	.132	.132	.132	.131	.131	.130	
Checker spot butterfly. ( <i>Melitaea</i> sp.) Pupa .....										
Tent caterpillar moth. ( <i>Clisocampa</i> sp.) Pupa ...	.291	.280	.270	.264	.254	.248	.225	.203	Moth issued.	
Mourning-cloak butterfly. ( <i>Euvanessa antiopa</i> ) Pupa..	.305	.295	Dead.							

\*Pupa was lifted with fingers at time of weighing instead of with forceps; the little moisture adhering would produce the difference in weight.

From this table it is apparent that the silken cocoon loses a very small amount, about 4 per cent., of its weight in the first day after its completion, and then loses no further weight; that the pupa loses weight slightly but persistently and steadily from day to day throughout its entire duration, the total loss amounting to about 14 per cent.; and that the pupæ of three other lepidopterous insects, namely, the tent caterpillar (*Clisiocampa* sp.), checker-spot butterfly (*Melitaea* sp.), and mourning-cloak butterfly (*Euvanesa antiopa*) also steadily lose weight from day to day, this loss being very considerable in two of these species, viz., about 35 per cent. in the case of one and 65 per cent. in the case of the other.



# THE LOCATION OF THE CHICK EMBRYO UPON THE BLASTODERM.

BY

FLORENCE PEEBLES, Ph.D.

WITH 2 PLATES AND 15 FIGURES IN THE TEXT.

An experimental study of the avian egg has led me to examine the following points:

1. The location of the embryo in the material of the unincubated blastoderm.
2. The direction of growth before, and after the appearance of the primitive streak.
3. The origin of the material from which the later embryo arises.

According to Kopsch<sup>1</sup> this third point has been definitely settled. He concludes that nearly all of the embryo develops from the primitive streak. I quote his own words: "Somit entsteht der Embryo, mit Ausnahme des praechordalen Teils, des Kopfes, durch Umwandlung des Primitivstreifens."

I<sup>2</sup> have already mentioned some experiments, and will describe others, in the following pages, which seem to prove that only the trunk and caudal regions of the embryo arise from the material of the primitive streak.

The methods used by Assheton, myself, and Kopsch are practically the same, and therefore require little explanation. I have

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<sup>1</sup>Kopsch, Fr. Ueber die Bedeutung des Primitivstreifens beim Hühnerembryo. Leipzig, 1902.

<sup>2</sup>Peebles, F. A Preliminary Note on the Position of the Primitive Streak, and its Relation to the Embryo of the Chick. Biolog. Bulletin, Vol. IV, No. 4, 1903.



again used the method described in my earlier work.<sup>1</sup> A small window was made in the shell just above the blastoderm, and the operation performed, after which the opening was closed by a piece of shell, sealed with strips of the shell membrane. All instruments used in the experiments were carefully sterilized, and the shells of freshly opened eggs used for closing the windows. The loss of eggs through infection was small. In most of the experiments one egg in each set was opened and then sealed again, without operating upon it, in order to have a check with which to compare the eggs upon which experiments were made. In this way it was possible to determine roughly, after further incubation, whether abnormalities were due to opening the egg or to the operation performed upon the blastoderm. In general it was found that the development of eggs in which windows were made was delayed about two to four hours.

#### I. THE LOCATION OF THE EMBRYO IN THE MATERIAL OF THE UNINCUBATED BLASTODERM

In 1896, Assheton<sup>2</sup> described some experiments that he made on the unincubated blastoderm of the chick. Sable hairs were inserted at various points and their position determined after periods of incubation varying from eighteen to forty hours. Assheton proved that Duval's<sup>3</sup> theory of the formation of the primitive streak is incorrect, that instead of forming by the concrescence of the posterior margin of the blastoderm, the primitive streak appears in the region of the unincubated blastoderm which lies between the center and the posterior margin of the area pellucida. I have repeated Assheton's experiments, making the injuries with a hot needle instead of a hair, without removing the egg from the shell. The results agree with those of Assheton, as the following experiments show:

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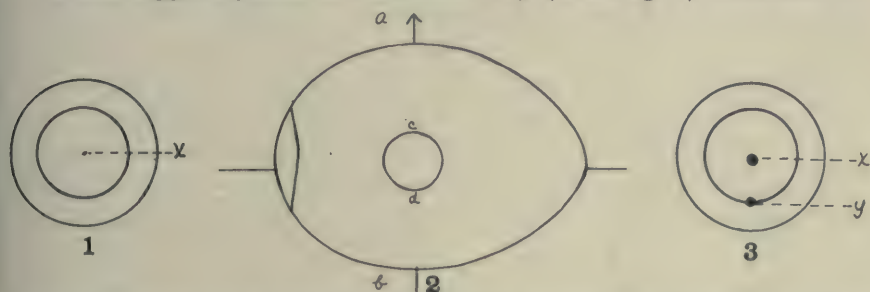
<sup>1</sup>Peebles, Florence. Some Experiments on the Primitive Streak of the Chick. *Archiv. für Entwicklungsmech. der Organismen*. VII Band. 1898.

<sup>2</sup>Assheton, R. An Experimental Examination into the Growth of the Blastoderm of the Chick. *Proceedings of the Royal Soc.*, Vol. 63, 1896.

<sup>3</sup>Duval. De la Formation du Blastoderme dans l'Oeuf d'Oiseau. *Annales des Sciences Naturelles, Zoologie*, Vol. 18.

*Experiment I.* A small window was made in the shell of an egg a few hours after it was laid. The blastoderm measured 2.8 mm. in diameter and the area opaca and area pellucida were faintly defined. A hot needle (No. 12) was inserted in the center of the blastoderm (Text-fig. 1, *x*) and quickly withdrawn. The shell was sealed and the egg put in the incubator, the temperature of which varied from 37°–39° Centigrade. At the end of twenty hours the egg was opened, and the blastoderm killed, removed and stained. The primitive streak was clearly defined (Pl. I, Fig. 1) extending from the posterior margin of the area pellucida to the point of injury (*x*). The cells around the wound seemed greatly increased in number and showed evidence of forward growth which must have been stopped by the injury.

Other eggs, injured in the same way (Text-fig. 1) were left in



the incubator for a longer period, from thirty to forty-eight hours. Pl. I, Fig. 2 is a surface view of one of these embryos after forty-eight hours' incubation. The embryo is well developed, fourteen pairs of somites are present, and the heart is forming. The injured area lies dorsal to the heart on a level with the anterior somites. The brain region has failed to develop.

From Assheton's results, and from these just described, we must conclude that the primitive streak and the greater part of the later embryo form from that region of the unincubated blastoderm which lies behind the center, between it and the posterior margin of the area pellucida. The question arises whether or not the posterior margin of the area pellucida is a fixed region in all eggs, and what the relation of the long axis of the embryo is to the long axis of the shell. In Text-fig. 2, an egg is represented

as opened above the blastoderm. The air chamber, which lies in the blunt end of the shell, is at the left, and the pointed end at the right. The chalazæ extend on each side of the yolk in the long axis of the shell. In this position the blastoderm may be divided into right and left halves, the arrow *a-b* indicating the median plane of the bi-laterally symmetrical embryo. We then speak of the region *c* as the anterior border, and *d* as the posterior border of the blastoderm.

Assheton, in another series of experiments, has made two injuries in the unincubated blastoderm (Text-fig. 3) one in the center (*x*) and the other in the posterior border (*y*) of the area pellucida. He found that the primitive streak appeared later between these two injuries, and he concluded from this that the point (*y*) marks the posterior end of the embryo.

In order to distinguish the region of the primitive streak from the rest of the area pellucida, I shall call it the radius *x-y*. This radius with the corresponding one anterior to the center make the diameter which represents the median longitudinal axis of the embryo. In order to determine the constancy of the occurrence of the embryo in this position I have kept the record of 100 eggs. The eggs were taken from the nest on the same day that they were laid. They were placed in the same position in a basket from which they were transferred to the incubator. After incubation for eighteen to forty-eight hours the embryo in every fertile egg, with the exception of two, was found in the median line (Text-fig. 2 *a-b*). The two exceptions are shown in Text-figs. 4 and 5. The first embryo (Text-fig. 4) was incubated eighteen hours. At the end of this time the primitive streak had formed, but instead of lying on the radius *x-y* it extended from the center to the right side of the blastoderm and was bent towards the posterior margin. The second egg (Text-fig. 5) was incubated for a period of twenty-eight hours. The normal embryo lay at right angles to the line *a-b*. In both of these eggs the chalazæ were found in abnormal positions, and the yolk membrane was wrinkled in many places, showing that the yolk had been abnormally twisted in its passage through the oviduct.

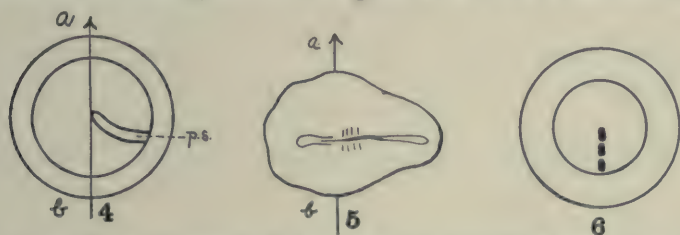
After I had discovered that the position of the normal embryo, when undisturbed by twisting or shaking, is constant, I deter-



mined to find out, if possible, whether the embryo would form on any other part of the blastoderm if development on the radius  $x-y$  was prevented.

*Experiment II.* The blunt end of the egg was held in the left hand so that the blastoderm lay on top of the yolk; a small window was made immediately above it, and a series of injuries were made with a hot needle in the radius  $x-y$ . The number of injuries was dependent upon the size of the area pellucida. Usually there is space enough to insert the tip of a No. 12 cambric needle in three places between  $x$  and  $y$  (Text-fig. 6) before all the cells are destroyed.

At the end of eighteen hours the eggs were killed, but no trace of primitive streak in another region was found. About 60 per cent. of the blastoderms showed a large hole where the area pellucida had stretched apart in the growth of the blastoderm. No



evidence of the formation of the embryo around the margin of the hole could be found.

The experiment was repeated and the eggs were incubated from thirty to forty hours. An examination of these embryos showed no development around the margin of the wounded area, but in front of it and posterior to it some development had taken place. In Pl. I, Fig. 3, a surface view of an embryo of forty hours' incubation is given. The brain is abnormal, but shows no lack of material, the notochord is present, but greatly reduced in length. There is some trace of the heart lying on each side of the notochord, but back of it none of the embryo has formed. There is no evidence of growth of the area pellucida in a posterior direction, but anteriorly it is the normal size and shape.

Another embryo incubated thirty hours is shown in Pl. I, Fig. 4. In this embryo no brain developed but growth from the heart



region caudad is evident. The injured area ( $w$ ) is surrounded by thickened ridges and back of the hole made by the wound the notochord is present. Behind the notochord lies the posterior end of the primitive streak. No mesoblastic somites are present.

The object of these experiments was to prevent development along the radius  $x-y$  by killing the cells in the region where the primitive streak develops. In this way it was hoped that the primitive streak might be formed in some other part of the area pellucida. The results show very clearly that no other part of the blastoderm is capable of forming the primitive streak. They also show that the region of the unincubated blastoderm along the radius  $x-y$  is the region from which the mesoblastic somites develop, *i. e.*, the trunk region of the embryo. From these experiments it seems evident that the position of the embryo upon the blastoderm is determined before the egg has been incubated, and probably before segmentation is completed, for some of the eggs which I used were operated upon within two hours from the time that they were laid.

## II. THE DIRECTION OF GROWTH OF THE EMBRYO BEFORE AND AFTER THE FORMATION OF THE PRIMITIVE STREAK.

Marshall<sup>1</sup> describes the growth of the blastoderm from the beginning of incubation as follows: "After incubation has commenced, the blastoderm spreads rapidly, retaining its circular shape. By the end of the first day of incubation it is about the size of a sixpence, and by the end of the second day it has extended nearly half way round the egg."

According to Duval<sup>2</sup> the edge of the blastoderm advances over the egg at every point except at the posterior margin, and the edges on each side of this point meet each other in the middle line to form the primitive streak ("plaque axiale"). Assheton's<sup>3</sup> experiments have proved, however, that the growth is symmetrical as Marshall states.

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<sup>1</sup>Marshall. *Vertebrate Embryology*.

<sup>2</sup>Duval. *Loc. cit.*

<sup>3</sup>Assheton. *Loc. cit.*

While the margin of the area opaca is symmetrical, that of the area pellucida is not. During the first few hours of incubation the two areas increase uniformly, but towards the fifteenth hour the area pellucida begins to extend posteriorly, the anterior region remaining spherical in outline.

*Experiment I.* The uniformity of growth in the anterior half of the blastoderm can be seen in the following experiment. The unincubated blastoderm was injured at three points (Text-fig. 7,  $x$ ,  $p$  and  $o$ ); the needle was inserted at the center ( $x$ ) at the middle point of the anterior margin ( $p$ ) and at the right margin of the area pellucida ( $o$ ). The injuries in the margin were at equal distances from the center. After eighteen hours' incubation the distance between  $x$  and  $p$  was the same as the distance between  $x$  and  $o$  (Pl. I, Fig. 5) showing that the lateral and anterior growth were the same. The primitive streak was formed, but its posterior end ( $y$ ) was much further from  $x$  than  $x$  was from  $p$ , while the distances before incubation were equal.

The results of earlier experiments<sup>1</sup> led me to believe that the region immediately in front of the primitive streak represents an area of rapid growth, because an injury made in this region did not affect one structure alone, but disturbed the organ covering a large area. This is also true when the center of the unincubated blastoderm is killed (Pl. I, Fig. 2).

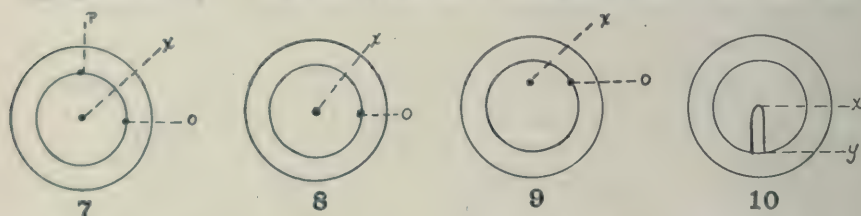
*Experiment II.* In order to determine the extent of growth in an interior direction from the center of the blastoderm I injured a point in the center (Text-fig. 8,  $x$ ) and one on the same level at the side ( $o$ ). The eggs were incubated thirty-six to forty hours. Pl. I, Fig. 6 is a surface view of an embryo at the end of thirty hours. The injury in the center of the blastoderm produced great disturbance in the development of the embryo anterior to the heart. No forward growth took place in the median line. The wound ( $o$ ) at the side, which did not move forward, is at a level with the anterior somites. The normal growth of the margin of the area pellucida on the side of the injury did not take place. The margin is irregular and a peculiar rod of cells extends from the marginal wound to the median line of the embryo.

<sup>1</sup>Peebles. *Loc. cit.*

*Experiment III.* In another set of experiments the two injuries were made about .5 mm. further forward (Text-fig. 9, *x* and *o*). The position of the injuries after forty hours is seen in Pl. I, Fig. 7. The wound at the side (*o*) has advanced with the growth of the blastoderm but the wound (*x*) in front of the somites has prevented the formation of the head, and the embryo is reduced in length anteriorly, the trunk and caudal regions are about the normal length.

From these experiments it seems evident that the region in front of the middle point of the area pellucida is the seat of active growth in an anterior direction.

*Experiment IV.* In order to determine the extent of growth posteriorly, two injuries were made, one in the center of the unincubated blastoderm and the other in its posterior margin (Text-fig. 3, *x* and *y*). The embryos were incubated thirty-six hours to



two days. They developed somites and medullary folds in the area between the wounds. Pl. II, Fig. 8, represents a surface view at the end of thirty-six hours. Notochord and somites have developed between the two wounds. The actual distance from *x* to *y* before the experiment was 1 mm. After incubation it was 3 mm. showing an increase in length of only 2 mm. The normal embryos at this age measure 4 mm. from heart to caudal end.

The results of these three sets of experiments show that the embryo may be greatly reduced in length by preventing growth anteriorly with the wound *x* and posteriorly with the wound *y*, and that the area pellucida grows less rapidly at the sides than in the median line.

Up to this time the experiments which I have described have been made upon the unincubated blastoderm. The change in the size and the shape of the area pellucida is comparatively



slight before the appearance of the primitive streak when the area becomes pear-shaped.

Kopsch<sup>1</sup> has found that when two wounds are made in an embryo of twenty-four hours' incubation, at a distance of 2 mm., one at the anterior, and the other at the posterior end of the primitive streak, the embryo does not reach its normal size in later development. The entire body is much shortened, and lies between the two wounds. I have repeated this experiment, and have obtained the same result, the primitive streak, in the eggs upon which I have worked, is much longer (3 to 3.5 mm.) in a twenty-four hour chick, and the anterior end is no longer visible, the head process and the notochord are present.

Another series of experiments was made by Kopsch when the primitive streak measured about 4 mm. A series of five injuries, at 1.5 mm. spaces, were made along the side of the primitive streak, and parallel with it. The embryos were incubated fifty and one-half hours, and at the end of this time the regions of the five wounds were located. Growth in length was greater in the region *back* of the anterior end of the primitive streak than it was in front of it.

I have already described experiments which I have made upon the primitive streak and have tried to show that the anterior end of the primitive streak of sixteen to eighteen hours represents the region of the later embryo which lies back of the heart between the anterior somites.

*Experiment V.* These experiments were repeated with some modifications. Instead of injuring the anterior end alone, a second wound was made at the posterior end (Text-fig. 10, *x* and *y*). The embryo at the time of the operation was from sixteen to eighteen hours old. After forty hours a normal embryo developed but instead of extending posteriorly to the usual length it was shortened 2 mm. Another egg injured in the same way (Text-fig. 10) developed into an interesting embryo (Pl. II, Fig. 10). The posterior wound (*y*) healed so that no trace of it could be discovered, but the anterior wound (*x*), through the further

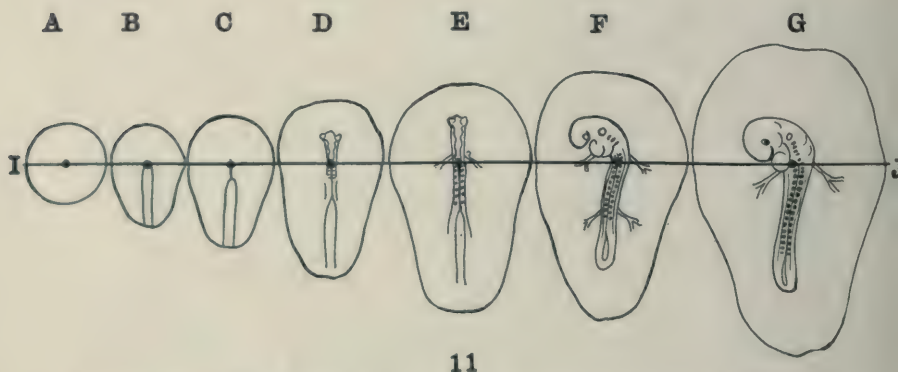
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<sup>1</sup>Kopsch. *Loc. cit.*



growth of the embryo, was left on one side. The only disturbance evident was in the medullary folds and somites on the side of the injury.

*Experiment VI.* A wound at the posterior end of the primitive streak is alone sufficient to shorten the embryo caudad. In Pl. II, Fig. 11, a surface view of an embryo of forty hours' incubation is shown. The wound (y) was made at the posterior end of the primitive streak of eighteen hours. Fourteen pairs of somites are present, and the embryo measures 3 mm. from the heart to the anterior border of the brain. This region is normal, but growth in a posterior direction has been stopped, by the wound, and the length is reduced 1.5 mm.



11

*Summary.* The results from these experiments show that in the formation of the third-day chick neither head nor tail region can be taken as fixed points, indeed no one point on the blastoderm can be said to be fixed. In the series of diagrams (Text-fig. 11, A-G) I have indicated, in a schematic way, the method of growth from the beginning of incubation until the third day. The growth of the area opaca is symmetrical therefore it is not included in the diagram. The line I-J represents the plane dividing the unin-cubated blastoderm into anterior and posterior halves, and passes through the region in the older embryos which corresponds to the middle point of the area pellucida before incubation. From this point growth proceeds in all directions in the plane of the blastoderm. The growth from the first to the twelfth hour is sym-

metrical. From the twelfth to the eighteenth hour the area pellucida increases in length posteriorly. From the eighteenth to the twenty-fourth hour growth continues posteriorly and also proceeds in an anterior direction. From the end of the first day to the end of the second day it advances from the heart in both directions, more rapidly caudad than cephalad. After this time the tail and head are folded off from the surface of the blastoderm.

### III. THE ORIGIN OF THE MATERIAL FROM WHICH THE LATER EMBRYO ARISES.

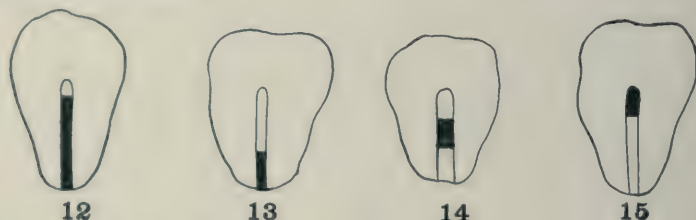
I have already spoken of Kopsch's conclusions as to the material from which the embryo arises, so that I shall merely mention my own results. If, as Kopsch says, the primitive streak represents the entire embryo with the exception of the pre-chordal head region, then the destruction of definite areas of the primitive streak should result in a failure to develop the parts which arise from the injured area.

*Experiment I.* The first experiment consisted in destroying all of the primitive streak except its anterior end (Text-fig. 12). This operation is very likely to kill the entire embryo as injury to so large an area usually results in a spreading apart of the margins of the wound. The further development of an embryo injured in this way may be seen in Pl. II, Fig. 12. The embryo is abnormal, but shows structures which indicate that when deprived of all of its material except the anterior end the primitive streak gives rise to the first few pairs of somites; and that the brain and notochord develop. The somites are much thinner than in the normal embryo.

*Experiment II.* In another series of experiments the posterior third of the primitive streak was destroyed (Text-fig. 13). The destruction of this region resulted in an embryo (Pl. II, Fig. 13), in which the entire caudal region was abnormal. The heart and brain, which are not represented in the figure, were normal, and fifteen to eighteen pairs of somites were formed in the anterior trunk region. This result agrees with Kopsch's view that the posterior third of the primitive streak represents the caudal region of the embryo from the twentieth somite to the posterior end.

*Experiment III.* In a third series of experiments the middle part of the primitive streak was killed (Text-fig. 14), leaving some of the material in front, and some back of the wound. According to Kopsch, in the later embryo the region from the first to the twentieth somites should be lacking.

Nearly all of the embryos which I operated upon, in this way, were so greatly disturbed by the wound that all development was checked. In Pl. II, Fig. 14, a surface view of the body region of one of the embryos which developed further is shown. The brain and heart were normal, therefore they are not included in the figure. Posteriorly the wound (*w*) stretched apart, but anteriorly medullary folds and ten or twelve pairs of somites are present. This result indicates that at least ten or twelve pairs of the first twenty somites come from the material in the anterior third of the primitive streak.



*Experiment IV.* Finally, the anterior third of the primitive streak was killed (Text-fig. 15). After further incubation the embryo developed a normal brain and heart in front of the wound. The trunk region (without the brain and heart) of one of these embryos is shown in Pl. II, Fig. 15. Back of the wound (*w*) eleven to fourteen pairs of somites are present. By comparison with normal embryos of the same age I conclude that these somites represent approximately, the tenth to the twentieth pairs, therefore all of the somites between the first and tenth pairs have been destroyed by injuring the anterior one-third of the primitive streak. The notochord is also lacking in these embryos.

It is evident from these results that the primitive streak of eighteen hours represents the material from which the trunk and tail regions of the later embryo develop; that the posterior third of the primitive streak represents the region back of the eighteenth



pair of somites, the middle third represents roughly, from the twelfth pair to the eighteenth, while the anterior third supplies material for those structures which lie *between* the heart and the twelfth pair of somites, but *does not* include the chordal region of the brain.

#### SUMMARY AND CONCLUSIONS.

1. The central point of the unincubated blastoderm represents the anterior end of the primitive streak, and later, the region just back of the heart; therefore, the greater part of the embryo develops in the posterior half of the blastoderm.

2. The region midway between the center of the unincubated blastoderm and its anterior border represents the head region of the later embryo.

3. The position of the embryo on the area pellucida is fixed. The long axis of the future embryo divides the unincubated blastoderm into right and left halves and a line drawn through the blastoderm in the long axis of the shell divides it into anterior and posterior halves.

4. Destruction of the material of the unincubated blastoderm between the center and its posterior margin does *not* result in the formation of the primitive streak on any other radius.

5. The growth of the blastoderm is uniform up to the eighth to tenth hour, and this uniformity is preserved in the later growth of the area opaca, but from the tenth hour the area pellucida begins to grow more rapidly in a posterior direction, then later it advances anteriorly until it assumes an oval form. Up to the third day the region immediately back of the heart (the anterior end of the early primitive streak) is the center of growth in all four directions, anteriorly, to the left, and to the right, and to a much greater extent posteriorly.

6. Injury to the center and posterior margin of the unincubated blastoderm results in a shortened embryo.

7. Injury at the posterior margin alone will shorten the embryo by preventing growth in a posterior direction.



8. Neither head nor tail region of the embryo can be taken as fixed points, the growth at each end proceeds until the head and tail become folded off from the blastoderm.

9. After destruction of all of the material of the primitive streak except its anterior end a small embryo with eight to ten pairs of somites develops.

10. The posterior third of the primitive streak furnishes the material for the caudal region of the later embryo. The middle third represents the trunk region, and the anterior third that part of the embryo which lies between the heart and the tenth to twelfth pairs of somites. The material of the primitive streak does not enter into the formation of the brain.

The Woman's College,  
Baltimore, June 1, 1904.

EXPLANATION OF PLATES.

PLATE I.

Fig. 1. Blastoderm 20 hrs. old.  $x$ , point of insertion of hot needle before incubation.

Fig. 2. Ventral view of embryo 48 hrs. old. Injury made in center of unincubated blastoderm lies back of heart. Brain undeveloped.

Fig. 3. Surface view of forty-hour embryo in which the material along the radius  $x$ - $y$  had been killed. Heart, brain and notochord are present.

Fig. 4. Embryo 30 hrs. after operation described for Fig. 3. Heart and posterior body region present.

Fig. 5. Primitive streak 18 hrs. old. The three black areas indicate the positions of the injuries made upon the unincubated blastoderm.

Fig. 6. Surface view of embryo 36 hrs. old. The black areas indicate the wounds  $x$  and  $o$  made in the blastoderm before incubation.

Fig. 7. Embryo 40 hrs. old. The openings  $x$  and  $o$  indicate the wounds made in the blastoderm.

PLATE II.

Fig. 8. Embryo 36 hrs. old. The position of the wounds is indicated by the black areas  $x$  and  $y$ .

Fig. 9. Embryo 40 hours after injuries were made in the anterior and posterior end of the 18 hr. primitive streak.

Fig. 10. Embryo 12 hours older than that in Fig. 9 after the same operation.

Fig. 11. Forty-hour embryo in which an injury had been made in the posterior end of the primitive streak of 18 hrs. The black region ( $y$ ) indicates the wound.

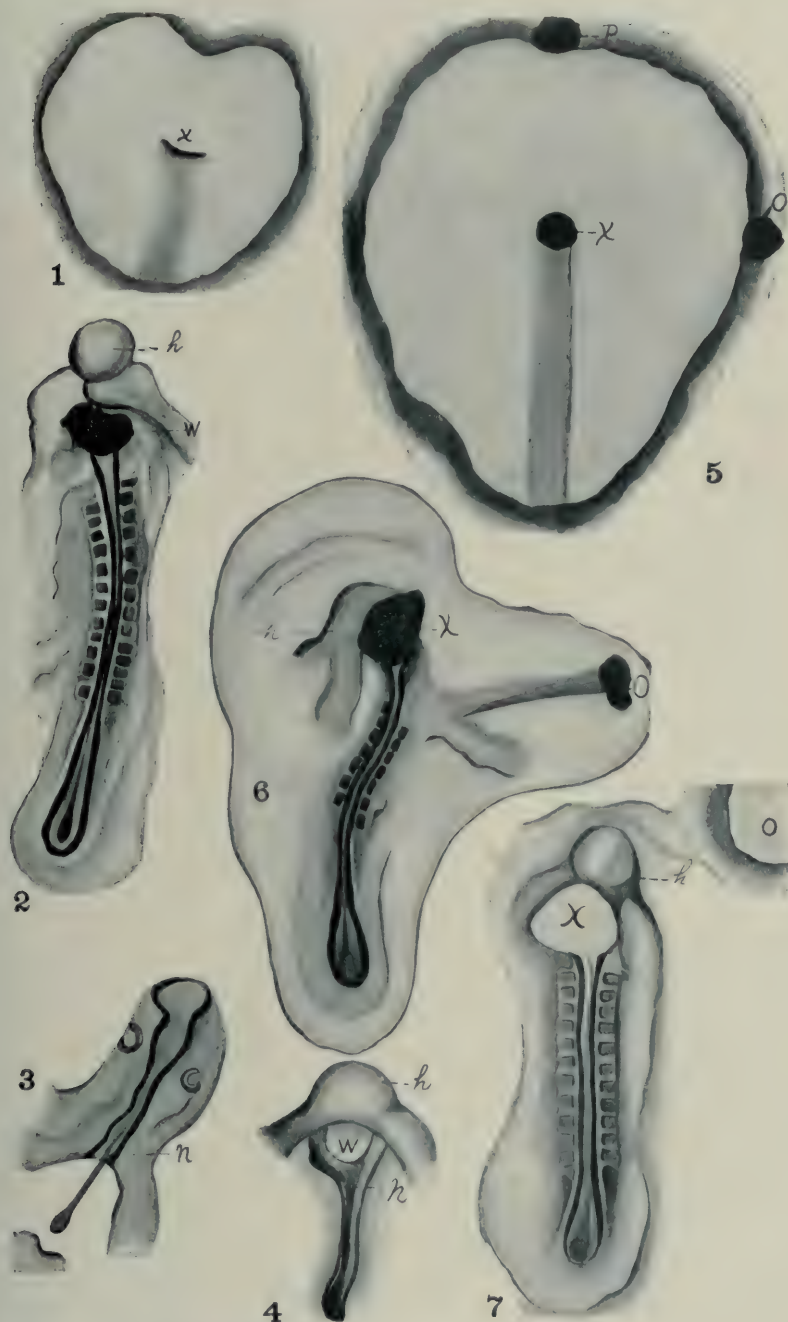
Fig. 12. Embryo incubated 36 hrs. after four-fifths of the material of the primitive streak was destroyed, leaving only the anterior end.

Fig. 13. Fifty-hour embryo in which the posterior third of the primitive streak ( $w$ ) was destroyed. The brain which was normal is not shown.

Fig. 14. Surface view of embryo 30 hours after the middle part of the primitive streak was destroyed. The normal brain is not shown.

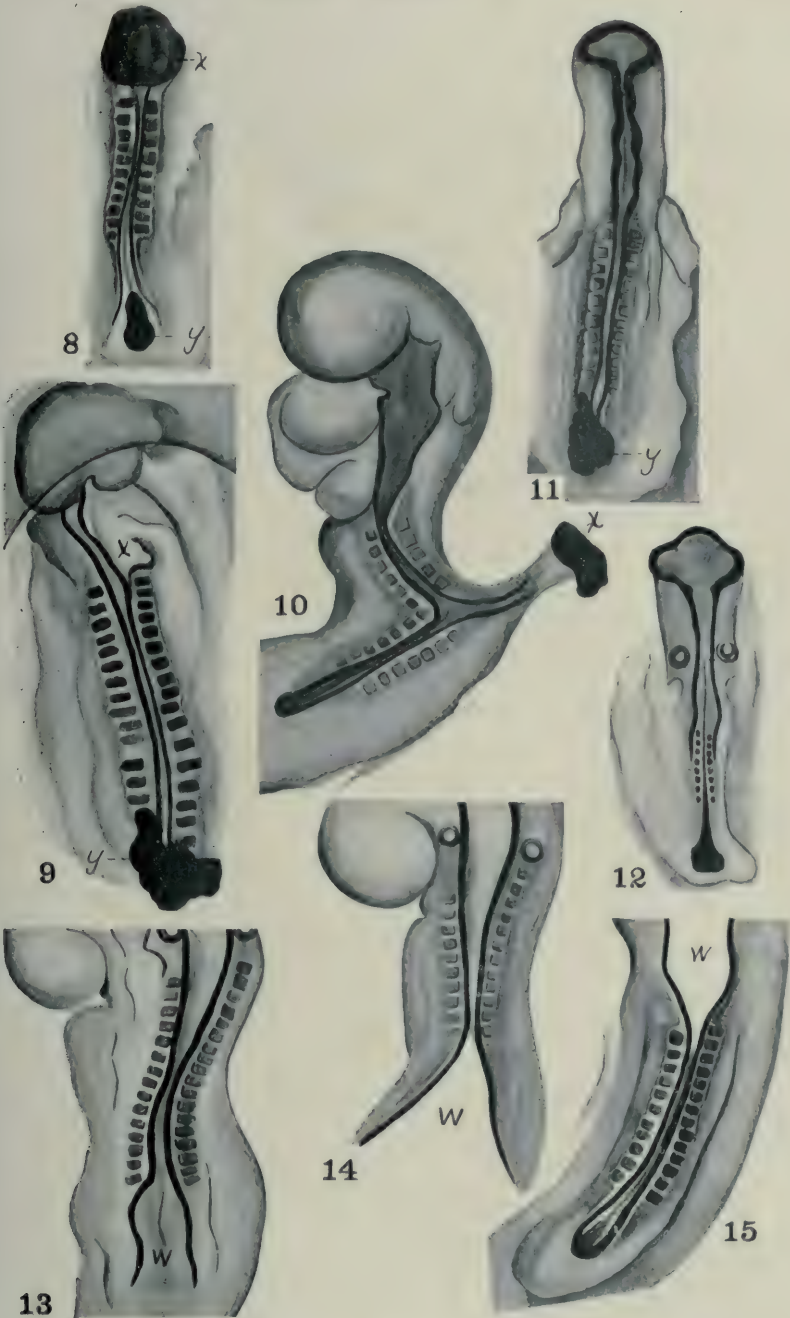
Fig. 15. Embryo of same age as preceding one. The anterior third of the primitive streak was destroyed. Heart and brain which are not given are normal.  $w$  in these figures indicates region of injury.













# REGENERATION OF HETEROMORPHIC TAILS IN POSTERIOR PIECES OF PLANARIA SIMPLICISSIMA.<sup>1</sup>

BY  
T. H. MORGAN.

WITH 20 FIGURES.

The regeneration of a heteromorphic head from the posterior end of short cross-pieces of *Planaria maculata*, Figs. 1-5, led me to examine *Planaria simplicissima*<sup>2</sup> in order to see if the same result could be obtained here when short cross-pieces of the worm were made. The regularity with which a heteromorphic head can be obtained in the latter species when the old head is cut off just behind the eyes, Fig. 10, led me to expect that short cross-pieces from the body would behave in the same way as do similar pieces of *Planaria maculata*. The results have proven, however, in part otherwise, for while heteromorphic heads do appear on short cross-pieces from the anterior regions of the worm, Fig. 11, none such develop from the posterior end of short cross-pieces from the more posterior regions of *Planaria simplicissima*. On the contrary these pieces regenerate a structure from the anterior cut surface that appears to be a heteromorphic tail, and another tail from the posterior cut surface, Figs. 12, 13. The result is a two-tailed and not a two-headed piece. In order to determine if the new anterior structure is really a tail, and not simply an undeveloped head, a number of experiments were carried out during the winter and spring of 1903-04.

Before describing the results certain general considerations must be spoken of that are intimately connected with the question

<sup>1</sup>The principal facts recorded in this paper were reported at the Christmas meeting of the American Zoölogical Society, 1903.

<sup>2</sup>This is the same species which, in my earlier papers, has figured as *Planaria lugubris*. Stevens has recently determined that this worm is *P. simplicissima*.



of heteromorphosis in regions posterior to the old pharynx. An important side light is thrown on the problem of axial polarity and heteromorphosis by these relations.

Cross-pieces of *Planaria simplicissima*, that are *not too short*, from the region between the head and the pharynx-chamber regenerate a head on the anterior and a tail on the posterior cut surface,

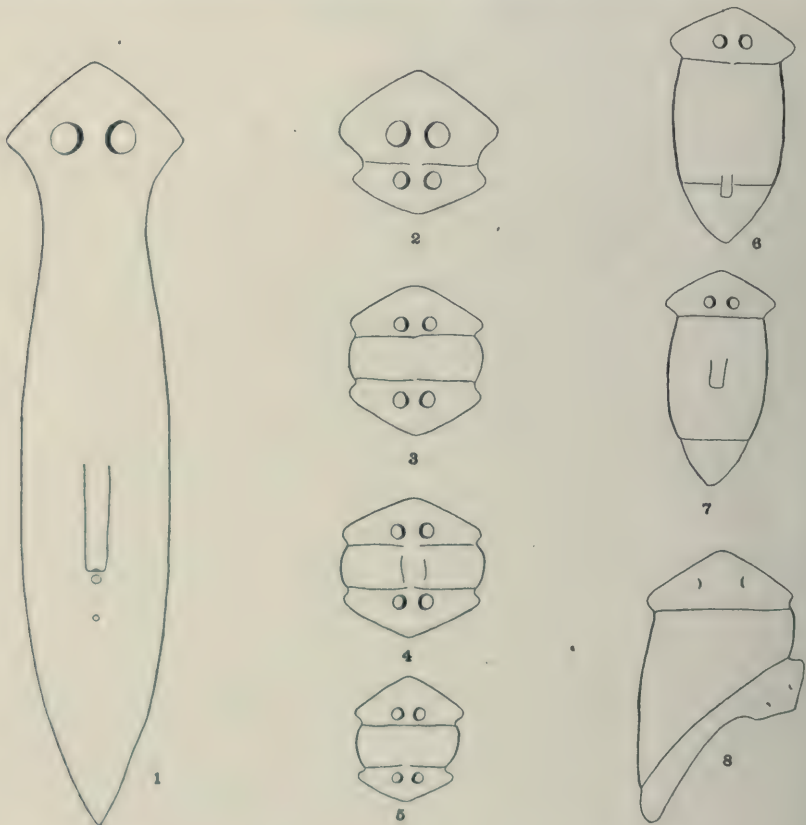


Fig. 14. The new pharynx is always situated at the posterior edge of the old material. Similar cross-pieces from the region of the pharynx-chamber also produce a head at the anterior end and a tail at the posterior end. The new pharynx develops in the middle of the piece in connection with the old chamber. Cross-pieces from the region behind the old pharynx also regenerate a head at

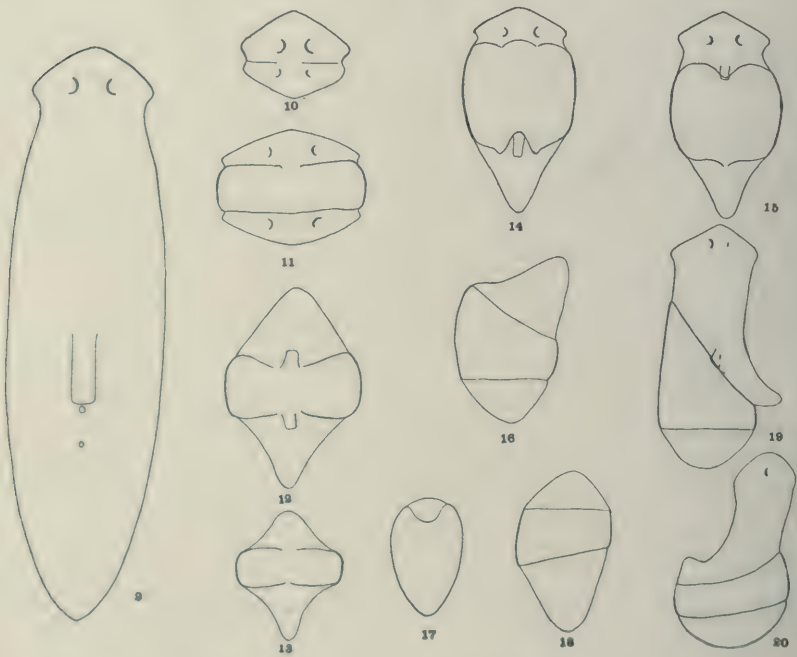
the anterior and a tail at the posterior end. The new pharynx always lies *at the anterior end* of such pieces, *i. e.*, at the edge of the old tissue, and therefore, as it were, in the posterior part of the new head that has developed, Fig. 15. It is this relation of the new pharynx to the old part that first demands especial consideration, for, at first sight it is not clear why the pharynx in these posterior cross-pieces should shift to the anterior end, and not lie, as in the more anterior pieces, at the posterior edge. If it did so it is obvious that it would appear in a region posterior to that in which the normal pharynx lies in the old worm, and it seems that this cannot take place. The posterior cut surface can form only that part of the tail that lies behind it in the old worm. The anterior cut surface can also produce all that lies in front of it in the old worm, including the pharynx, although the proportionate distances apart of the new structures may be at first very different from those in the adult or embryonic worm. We touch here on one of the fundamental questions of polarity to which I shall hope to return at another time.

If a heteromorphic tail were to develop on the anterior cut surface of a *short posterior cross-piece*, what should we anticipate in regard to its relation to a pharynx? Should we expect to find a pharynx in the new tail turned in the opposite direction, *i. e.*, pointing towards the tip of the tail? But if the new structure at the anterior end is a heteromorphic tail why should it develop a pharynx at all, since this never develops at the posterior end of cross-pieces from this region? Should we not rather expect a heteromorphic tail to behave in this respect in the same way as the orthomorphic structure? This appears to me to be the correct point of view and the results of experiment seem to bear out this anticipation.

Let us apply the same point of view to the regeneration of a pharynx in heteromorphic heads from cross-cut pieces of the anterior regions of the worm. It has been pointed out above that the new pharynx appears at the posterior end in case a tail develops at this end. Suppose, however, a heteromorphic head instead of an orthomorphic tail develops at the posterior end. It is clear, from our point of view, that no pharynx should develop, and I

have found that none such is present as a rule. In the few cases in which a pharynx appeared in the middle of the piece, the piece may have come from the region near to or through the pharynx-chamber of the old worm.

Turning now to the results of regeneration of *very short cross-pieces* of *Planaria simplicissima*, it was found that double headed pieces are sometimes obtained from the more anterior regions, Fig. 11, as in *P. maculata*, Figs. 2-5. When short cross-pieces



of *P. simplicissima* are cut off posterior to the pharynx-chamber a number of them produce a head at the anterior end and a tail at the posterior end, especially if they are rather long, Fig. 15; but short pieces and sometimes some of the longer pieces also produce quite often a pointed structure at the anterior end. In the majority of cases these anterior structures never develop into anything different, and resemble a tail in all respects. In a few cases, however, the pointed structure may become a head after some

time. The possibility that all the anterior pointed structures may be only undeveloped heads must therefore be given serious consideration. That they are not such in many cases is shown, I think, by the following facts. In the first place the movement of a piece in which the anterior head is undeveloped is very different from that of the two-tailed pieces, and reveals the nature of the new part; for, while the former crawls forward as do the pieces when first cut from the worm and as do those that develop an anterior head, the two-tailed pieces remain fixed in one place in the dish, and, if disturbed, fail to move in any definite direction. This is what we should anticipate if two tails were present working in opposite directions.

In the second place an orthomorphic pharynx appears as a rule when the head is delayed in its development while none such appears in the two-tailed pieces. In the third place the peculiar motion of the anterior end when it is irritated is similar to that of a tail and not like that of a head. Finally, the development in one case to be mentioned below of a two-tailed piece with pharynx in each tail shows, beyond a doubt, the possibility of the development of a heteromorphic tail in these worms.

After the short cross-pieces have been cut off for some time it is difficult to distinguish the anterior from the posterior end and to know which is the anterior heteromorphic and which is the normal posterior orthomorphic tail. In order to distinguish these apart, I cut off pieces obliquely at one end; in one set the anterior end being the oblique one, Fig. 16, in the others the posterior, Fig. 18. This necessitated increasing somewhat the length of the pieces and brought about in consequence an increase in the number of the pieces that regenerated a head at the anterior end. The record of one set of experiments of this sort is given here.

On May 7, twelve short cross-pieces were cut off just behind the pharynx. The anterior end of each was oblique. On May 20 there were alive three two-tailed pieces; the rest having died.

Twelve short tail-ends regenerated new tissue at the anterior end which in two cases at least appeared to be tails.

In another series twelve short cross-pieces were cut off behind the pharynx. The anterior end was square and the posterior end



oblique. Of the eight survivors all had pointed anterior and posterior ends.

The pieces just behind the last set (with oblique anterior ends) gave five two-tailed pieces, and one piece with head and tail.

The tail-ends of this set had the posterior tip cut off. The three that remained alive developed a pointed anterior end.

In another series like the last, the first pieces produced six two-tailed forms; the second six two-tailed forms; and the tail-ends five two-tailed forms.

In several other cases I allowed one end of the piece to close for two or three days before cutting it off. In this way the mortality of very short pieces, which is otherwise very great owing to their immediate disintegration, is lessened. Some of these pieces also gave two-tailed forms.

In only one case did I obtain a piece in which a pharynx was present in each tail, and in each turned outward toward the tip of the tail, as shown in Fig. 12. The exact location of this piece is, I am sorry to say, uncertain. It came, in all probability, from the region just behind, or including some of, the old pharynx region. I am inclined to think that the latter is the more probable location, since the cut may sometimes include somewhat more or less than is intended. The direction taken by the pharynges in these pieces shows beyond a doubt that one of the two tails is a heteromorphic structure, and this lends support to the interpretation that I have given to the other cases, in which there is no pharynx in the heteromorphic tail, and where none should be expected to be present on theoretical grounds.

Two other kinds of apparently heteromorphic structures have been met with in carrying out these experiments. In one case a piece of *P. maculata*, whose posterior end was cut off very obliquely, regenerated one head on the anterior cut surface and another also on the right side of the posterior cut surface, as shown in Fig. 8. A tail also developed on the posterior cut surface at the left side, which is also the more posterior end of this surface. In this case we must look upon the long edge of new tissue on the posterior surface as producing a head at one end and a tail at the other, very much as occurs when a longitudinal piece of the worm

is removed. The case recalls those in which the worm is split from the posterior end far forward and a head develops at the anterior end of the cut surface on one or on both sides. I have discussed the meaning of this case elsewhere.<sup>1</sup> In a strict use of the word heteromorphosis, as I have tried to use it for purposes of greater clearness, neither this case nor that of the split-worm can be looked upon as an example of axial heteromorphosis, since the result depends largely, apparently, on the new part alone without relation to the old, and the head and tail are orthomorphic from this point of view.

In some other cases in which the anterior end is very oblique, two structures appear on the anterior edge, as shown in Figs. 19-20. One of these is a head and the other appears from its structure and movements to be a tail. If so, the case is comparable to the last one, and shows the converse condition. Here the tail on the side of the anterior cut surface cannot be looked upon as an example of axial heteromorphosis. It is rather an orthomorphic structure, since it stands in this relation to the remainder of the new material on the anterior cut surface. Both cases, however, present something of a paradoxical relation.

The results described in the first part of this account recall certain conditions that I have recently described in connection with the regeneration of *Dendrocælum lacteum*. It had been shown by Lillie that posterior pieces cut off just in front of, or through, or behind the pharynx-chamber do not regenerate an anterior end. A histological examination of the anterior end of such pieces showed me that a certain amount of new tissue is formed at the anterior cut surface, and it was not apparent why the regeneration should not go further and produce a new anterior end. The results with *Planaria simplicissima* suggest, although they by no means prove, that the anterior part that regenerates in *Dendrocælum* may be a heteromorphic tail. For the present, however, I wish to leave this question open, until further work reveals the nature of the anterior part in this worm. There are some general considerations in connection with the problem of polarity

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<sup>1</sup>Regeneration. 1900.

and of heteromorphosis that may be very briefly touched upon at this time. Although I have not hesitated in earlier papers to speak of polarity as a factor in regeneration, I have always tried to be careful to state that we are really entirely ignorant in regard to its nature. When we see the polarity suddenly reversed in cases of axial heteromorphosis it appears that this ought to throw some light upon the nature of the factor itself, yet despite the numerous surmises that have been made of a material,—chemical, or electrical nature—we still remain totally in the dark as to what factors determine the stereometrical relations of the new part. The following facts appear, nevertheless, to have an important bearing on this topic, and while they do not offer an immediate solution of the problem, yet they may point in the direction in which an analysis may ultimately be undertaken.

In the more highly specialized forms the question of what regenerates appears, in part, to be connected with the nature of the material, or with the kinds of the material that give rise to the new cells, and the relation of direction is less apparent. The tail of a tadpole regenerates only a tail, even at its anterior end. The same appears to be true for the leg of the salamander from certain results that I have obtained, which are as yet unpublished. In the earthworm as shown by Morgan<sup>1</sup> and by Dimon<sup>2</sup> the regeneration of an orthomorphic head is connected with the presence of the anterior structures of the worm, while from the part containing the intestine—including by far the greater length of the worm—only a tail is, as a rule, regenerated, even from the anterior cut end. In these cases it appears that the nature of the material must decide the character of the new part, and the polar relations do not come conspicuously to the front, although that something of the sort still enters into the problem is shown by the slower rate, and, in some cases, by the less perfect form of the heteromorphic growth.

On the other hand, in less specialized forms the polar relations appear to play a more conspicuous rôle. In *Lumbriculus* a head

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<sup>1</sup>Anatomischer Anzeiger. Bd. 15, 1899.

<sup>2</sup>Journal of Experimental Zoölogy. Vol. I, No. 2, 1904.



may regenerate from an anterior cut surface, and a tail from a posterior cut surface throughout a very considerable region of the body. In planarians and in hydra similar facts are known. That the specification of the tissues or parts plays a rôle even in these cases is probable, as shown by the cases of heteromorphosis that I have described. To many writers it has seemed that the factor of polarity may be something in the nature of a crystallizing force—to use the nearest analogy at hand—a sort of perfecting or completing principle. Newer results have modified our ideas as to this form of explanation, if such an analogy can be called at all an explanation. The fact, for example, that in the earthworm and in planarians the new head may be very short in comparison to the part that is missing indicates that a completing force cannot be acting from the cut surface forwards, but whatever the nature of the factor it must in large part work from without (surface) inward (*i. e.*, toward the cut end). This point has been already urged by myself, and by Driesch.

It is very significant, I think, to find that in planarians the shortness of the piece is a factor that enters into the problem as to the character of the new part. I have suggested tentatively that this means that in *Planaria maculata* the tendency is stronger for the new structure to become a head than a tail, and that when the influence of polarity is removed a head appears on each end of short cross-pieces. In other worms, as in *Planaria simplicissima*, the tendency in certain posterior regions to produce a tail is stronger than that to produce a head, and two tails appear when the polarity is reduced or removed. Why should the length of the piece be so important a factor? Can it be that there is a greater difference, chemical or physical, between the two ends of a longer piece, so that a stronger polarity is present? In short pieces, from this point of view, the ends being near together are so much alike that the polarity is correspondingly reduced, and, under these conditions, the specification of the material of the old part is not sufficiently strong to determine the nature of the new part. These and many other equally obscure questions remain for future investigation to explain.





# BIOLOGICAL STUDIES ON CORYMORPHA.

## I. C. PALMA AND ENVIRONMENT.

BY  
HARRY BEAL TORREY.

WITH 5 FIGURES.

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- Introduction.
- I. Description of *C. palma*.
- II. Habitat; food.
- III. Activities of the polyp.
  - a. Muscular movements.
  - b. Geotropism; axial cells.
  - c. Locomotion; amœboid cells.
  - d. Circulation; cilia.
- IV. The young hydroid.
- Summary.

### INTRODUCTION.

This is one of a series of papers which deal with some of the phenomena of growth, differentiation and development in *Corymorpha*, from different points of view. The study of developmental mechanics has long since ceased to consist merely in an analysis of the development of egg and embryo. That regenerative development must be included goes without saying; and it is with the feeling that the normal activities not ordinarily considered in the category of developmental processes should be included also, that I have incorporated much of what may at first sight appear to be purely physiological material.

*Corymorpha* is an exceptionally attractive basis for such an investigation. In the first place, it combines remarkable powers of regeneration with a simple development from the egg, the non-

sexual origin of sexual individuals, and powers of movement that are unusual for a hydroid. In the second place, very little is known of its biology, and its value for experimental work has not yet been generally realized. No account of its regeneration has been published, with the exception of a brief reference to it in a former paper by the present author ('02, p. 41). Up to this time our knowledge of the egg-development has been based upon three stages described and figured by Allman ('71):<sup>1</sup> the sessile planula, the polyp with six proximal tentacles, and the polyp with sixteen to twenty. These Allman took for stages in the development of what he called "frustules," minute bodies cut off from the processes that develop near the base of the stem and really give rise to the filaments of the hold-fast. Had he seen the eggs on the medusæ in his aquarium, this pardonable error would not have been made. Agassiz ('62) and Allman ('63) have given brief accounts of the natural history of the hydroid and the development of the medusa.

These works, with several of a taxonomic character, and a recent paper by May ('03), comprise the scant publications on *Corymorpha* relating to the subject of this paper.

#### I. DESCRIPTION OF *C. PALMA*.

The nutritive polyps of *C. palma* are solitary. The stem may reach the length of ten centimeters, tapering gradually from a diameter of perhaps six millimeters, near the base, to a narrow neck which supports the hydranth. It is covered for about its proximal third with a thin, non-supporting layer of perisarc. Within is a solid axis of immense vacuolated cells. These have almost obliterated the cavity of the stem, which persists as a number of small, longitudinal canals lying immediately under the thin mesogloea, and usually made conspicuous by their green tinted walls.

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<sup>1</sup>A Monograph of the Gymnoblasic or Tubularian Hydroids. London, 1871. The section in this magnificent monograph devoted to *Corymorpha* is a reprint of Allman's paper in the Annals and Magazine of Natural History for January, 1863, p. 1, with but slight verbal changes and the addition of figures.

The hydranth has a single whorl of eighteen to thirty proximal tentacles with a spread of more than twenty-five millimeters. The proboscis, terminating in the mouth, is crowned with forty to sixty distal tentacles. Just within the proximal tentacles are several peduncles which bear numerous medusoid gonophores.

The stem is anchored by a tangle of filaments which arise on the longitudinal canals, beneath the perisarc, usually in pairs.<sup>1</sup>

## II. HABITAT; FOOD.

*Corymorpha palma* is a semi-tropical species, dwelling farther to the south than any of the other North American species of the genus. It has been found as yet only in two localities: in San Diego and San Pedro harbors, both on the southern coast of California. It lives under similar conditions in both places. At San Diego it was found in a slough near the mouth of the harbor, on a muddy bottom which was exposed at mean low water. At San Pedro it has flourished at various points in the harbor, always, however, on muddy flats. It occurs usually in definitely circumscribed patches, which change their position apparently with much caprice from year to year. A favorite location is along some small stream that drains the mud flats as the tide ebbs.

Copepods are numerous on the mud, which often carries patches of green composed of diatoms and other chlorophyll-bearing protista. All of these organisms seem to serve as food for *Corymorpha*, though the copepods form the staple article of diet.

## III. ACTIVITIES OF THE POLYP.

*Corymorpha* captures crawling diatoms and copepods by bending its column in a half circle and sweeping the sand with its tentacles. Floating organisms are caught when the column is in its usual erect position, with proximal tentacles fully extended. The oral tentacles are almost always active, bending restlessly now outward, now inward, now moving simultaneously, now independently. The proboscis is extremely mobile, capable of lengthening into a narrow stalk or contracting into a sphere, or

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<sup>1</sup>For a diagnosis of the species, see my paper just referred to.



turning inside out, or carrying the mouth with its prehensile tentacles to the bases of the proximal tentacles on all sides. The proximal tentacles are comparatively quiet. For minutes at a time they may be held in full extension, motionless, curved gracefully back from the proboscis, on a vertical stalk. Now and then one may twitch toward the mouth. Occasionally all may wave inward together, grasping the proboscis tightly. The points of the tentacles may be, but usually are not, carried directly toward the mouth.

#### a. Muscular Movements.

So far as I am aware, the reactions of hydroids (with the exception of *Hydra*) to different sorts of stimuli have never been studied. Medusæ, on the contrary, have been the subjects of extended investigations by Romanes ('76, '77), Eimer ('78), whose paper I have not seen, and Nagel ('93, '94). In certain respects, *Corymorpha* and some of the craspedote medusæ (*Carmarina*, *Sarsia*) respond similarly to similar stimuli. For instance, the proboscis of each may move toward a point of stimulation not on it; and increasing the stimulation of a tentacle may increase the number of tentacles taking part in the response, and leads finally to the contraction of the body of the animal. *Hydra* and *Corymorpha*, however, resemble each other more closely in their responses than either resembles a medusa. In general, similar structures respond similarly, but the tentacles of neither *Hydra* nor *Corymorpha* react to odorous substances, while according to Nagel ('93), the tentacles of *Carmarina hastata* do. Such exceptions, coupled with obvious differences in structure and habits between polyp and medusa, make it necessary to treat each case individually.

The large size of *Corymorpha* makes it an unusually favorable object for experimentation in this direction. Experiments with mechanical, chemical and thermal stimuli brought out the following facts:

*Mechanical Stimuli.* Each proximal tentacle responds to a touch or pinch from forceps by contracting in the same direction with the same strength, whether the stimulus be applied at the

base or the tip, on the oral, aboral or lateral surfaces. The response is always a bend inward, never outward. In this respect it differs from the tentacular responses in some anemones (*Cribrina*, *Sagartia*), where a tentacle commonly reacts to a slight touch by bending sharply at and toward the point stimulated. This reflex is clearly advantageous to the anemone, which it enables, to a limited extent, actually to pursue its prey. It is supplemented by another. As soon as the tentacle, which is adhesive, seizes the stimulating object, it contracts, carrying its capture to the mouth, over which it bends. Then, by means of the cilia with which the tentacle is covered, the object, at least if available for food, is swept off the end of the tentacle and dropped upon the lips.

While the general direction of the movement of the tentacles does not vary, the intensity of the contraction varies with the intensity of the stimulus. A touch or slight pinch produces a waving of the tentacle toward the proboscis, though without reaching it; and the tip of the tentacle is not directed toward the mouth. A stronger stimulus may cause the tip to touch the distal tentacles, may even cause the tentacle to coil against the proboscis.

When the stimulus reaches a greater intensity, it may induce simultaneous movements in several or all the proximal tentacles. Before this point is reached, however, it is able to set up movements in the distal tentacles and proboscis. If a proximal tentacle contracts, an effect is often evident among the distal tentacles, even though the proximal tentacle has not touched them. This effect is manifested either by a simultaneous downward movement or an indeterminate waving of all the tentacles, or by a downward motion of a few nearest the tentacle stimulated.

These movements of the distal tentacles may occur without any apparent movement in the proboscis. If, however, the stimulation of the proximal tentacle is increased (occasionally a very slight stimulus is sufficient to produce the movement) the proboscis also may bend, carrying the mouth and distal tentacles toward the tentacle stimulated. This is a coördinated reflex of the same purposive aspect as the movements of the proboscis of the medusæ *Sarsia*, *Tiaropsis* (Romanes, '77) and *Carmarina*

(Nagel, '94) toward stimulated points on the sub-umbrella. While the excitation may be transmitted by means of the nerves of the tentacles and proboscis, certain facts indicate that the direct pull of the tentacle on the base of the proboscis serves at least to reinforce the impulse and aid in guiding the tentacles and proboscis in the proper direction. For example, the proboscis never bends until the stimulated tentacle contracts, although this contraction may be delayed half a second or a second after the stimulus is applied—an unusual reaction time; it does bend, however, immediately upon the contraction of the tentacle. Again, when a simple grip of the forceps does not cause a movement of the proboscis, the movement may be induced by adding to the tactual stimulus a definite tension stimulus by pulling the tentacle or preventing it altogether from shortening.

Not only may stimulation of a proximal tentacle be followed by movements of distal tentacles and proboscis, but by movements of the stem as well, which contracts strongly when the stimulation is vigorous. Only that part of the stem ordinarily contracts which is not invested with perisarc, though the latter is too thin to be an effectual hindrance to contraction in the basal region, which at times does shorten considerably.

When one distal tentacle is pinched, the response is similar to what occurs when the distal tentacles respond reflexly to a stimulation of a proximal tentacle; that is, several or all the distal tentacles may wave outward and downward together, or indiscriminately outward. In response to this stimulus, the movement is always away from, never toward the mouth; in this respect it is contrary to the direction of the movement of the proximal tentacles. After the first reaction, however, the tentacles may move actively and singly toward and away from the mouth. This is the characteristic reaction when the stimulation is prolonged. The presence of a large food organism in the proboscis cavity will cause such movements, which will persist until it has been entirely swallowed. They are only moderately efficient, for the outward movement of each tentacle is quite as strong as the inward movement, and the tentacle retains its hold on the captured organism for an instant only. They are indeed far less efficient



than the tenacious tentacles of anemones with their more definite movements.

When the proboscis is pinched at its base, it bends toward the point of stimulation, the distal tentacles waving. A pinch at any point, of a sufficient intensity, will induce characteristic movements of the distal and proximal tentacles and a shortening of the stem. If the stimulus is not too strong, only the proximal and distal tentacles in the vicinity of the point of stimulation will react simultaneously.

A slight stimulation of the stem may produce characteristic movements of both sets of tentacles. The effect is not related to the position of the point stimulated; none of the parts reacting appear to distinguish the direction from which the impulse comes. The stem may also shorten, even to half its original length. The shortening usually takes place in the distal naked portion. The proximal third, however, which is covered with perisarc, may also contract; the perisarc is very delicate and in no way interferes with this or any other movement of the stem.<sup>1</sup>

*Chemical Stimuli.* Several substances were used: flesh in the shape of pieces of the shore gastropods *Littorina* and *Acmæa*, and boiled ham; clove oil, alcohol and acetic acid. In no case did the meat juices have the slightest appreciable effect on the hydroid; the same may be said of the clove oil. Only when touched by a stream of strong alcohol or acetic acid from a pipette did tentacles or column respond; the acid killed the former almost instantly. This response is evidently of the tactile order—as when an irritating fluid is poured on the hand. Substances which have for our perception odors and flavors, appear to produce no reactions.<sup>2</sup>

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<sup>1</sup>The proportion of stem covered by perisarc is based on measurements of expanded individuals, under normal conditions. When a hydroid has been standing in the same water for a week or two, it usually becomes much attenuated, and the part of the stem invested with perisarc then often appears longer than the distal naked portion. Often the ratio of the covered to the naked portion of the stem may become that of the larva (Fig. 5).

<sup>2</sup>Loeb ('95) has already criticised the use of the words "olfactory" and "gustatory" to describe the reactions to chemical stimuli of animals of whose consciousness we are as ignorant as we are of the consciousness of the *Cœlenterata*.



*Thermal Stimuli.* A rapid rise in temperature of several degrees, caused by flooding the hydroid gently with warm water from a pipette, produced a general contraction of the same character as the response to a strong tactual stimulus.<sup>1</sup> Gradual changes in temperature affect both irritability and the rate of growth, increase of temperature resulting in increased irritability and more rapid growth, and *vice versa*; the limits, however, were not determined.

The reactions of *Corymorpha* to the various stimuli considered above may be summarized as follows: All parts of the hydroid are very sensitive to mechanical stimuli, irritating chemicals and abrupt increases of temperature. Proximity to odorous substances, especially flesh, which might serve as food, awakens no appreciable response until the substances are actually touched. Food organisms, therefore, are probably detected only when they strike the hydroid. The mechanism for capturing them is interesting on account of the definite but dissimilar responses of the two sorts of tentacles and the coördination exhibited in the activities of all the parts. The proximal tentacles with their great spread (which sometimes almost equals the length of the stem) serve as the chief means of advertising the presence of food and carrying it to the mouth. These functions are sufficiently well discharged by a movement in one direction only—toward the mouth; but the absence of the preliminary movement in the direction of the stimulus, which has been noted among the anemones, entails a certain loss of efficiency. This loss of efficiency is compensated for to some extent by the movements of the distal tentacles and the proboscis. The stimulus which causes the movements is in the great majority of cases liable to be applied to the proximal tentacles, on account of their relatively much greater spread. And apparently because of this, whether acquired by habit or selection, the first movements of the distal tentacles in response to direct or indirect stimulation are downward and outward, toward the proximal tentacles; that is, toward the *usual*

<sup>1</sup>This contraction is the result of muscular activity, does not concern the axial endoderm (to be especially considered later under Geotropism) and is not to be compared, therefore, with such growth processes as were shown by True ('95) to follow in radicles of seedlings, transference from water at 0°C. to water at 18°-21° C.

point of stimulation and away from the mouth. These movements, together with the tendency of the proboscis to bend toward the point of stimulation, carrying the distal tentacles with it, undoubtedly supplement the movements of the proximal tentacles in bringing food to the mouth, and raise the average of efficiency of the prehensile mechanism.

The contractions of the stem muscles, determining a limited range of movement for the hydranth, may be of advantage to *Corymorpha*. The rapid shortening of the stem following strong stimulation, however, can have no value as a part of the mechanism of prehension, nor does it have any apparent usefulness as a means of defense against predatory enemies.

*b. Geotropism; Functions of the Axial Endoderm.*

Up to this point we have been considering the effects produced by the contractions of muscles,<sup>1</sup> in tentacles, proboscis and column, under certain sorts of stimulation.

We may now consider another type of motor reaction induced by another sort of stimulus which appears ultimately to affect another tissue element. This is the tendency of the stem, in assuming its most characteristic attitude, to turn directly away from the center of the earth, by what seems to be a change in the turgidity of the axial endoderm cells incited by the stimulus of gravity.

It will be unnecessary to enter into an extended discussion of the phenomena of geotropism,<sup>2</sup> which are familiar to all. A few words will suffice for the purposes of this paper.

<sup>1</sup>There are both longitudinal (ectodermal) and circular (endodermal) muscle fibers in both proximal and distal tentacles, proboscis and column. As might be expected from their activities, the circular fibers in the proximal tentacles form a much weaker sheet than the longitudinal, except where each tentacle joins the body of the hydranth. There the circular fibers are aggregated into a strong bandlike sphincter, and there the tentacles are wont to break away from the hydranth under unfavorable conditions. Such a habit of casting the tentacles seems to be characteristic of certain anemones, notably *Bolocera*, and is accomplished by a similar sphincter.

<sup>2</sup>Davenport has distinguished between the responses of free and fixed organisms to gravity, following Schwartz in applying to the former the term "geotaxis," and applying to the latter the term "geotropism." With the facts which follow in mind, it will be difficult, I believe, to see any advantage in this distinction, and it has accordingly been disregarded.

Gravity affects both free and fixed organisms, and, so far as we are concerned with it, determines orientation, direction of locomotion, and direction of growth. In free organisms, orientation may or may not be accompanied by locomotion. Davenport has cited the infusorian *Spirostomum* as an organism which may belong in the latter category. In this case, orientation is finally due to the action of cilia with which the animal is clothed; if locomotion is associated with orientation here, it is very slight and inconspicuous. *Cerianthus*, whose negative geotropism was first considered by Loeb ('91), orients itself by means of muscular action. Though a free organism, it pursues a sedentary habit. The same tendencies are manifested by sand-dwelling anemones.

Among fixed forms may be considered (1) those which are attached aborally, but are also capable of some degree of locomotion, such as most of the anemones and the hydroid *Corymorpha*; (2) those which are permanently attached, such as most of the hydroids; and with these must be classed plants, especially seedlings. I have recently referred ('04) to the geotropism of the anemone *Sagartia davisii*, the orientation being accomplished, as in *Cerianthus*, by muscles. In the discussion of the geotropism of *Corymorpha* to follow, it will be shown that the orientation of the column is probably accomplished, not by muscles, but by means of growth processes comparable with the growth processes responsible for the orientation of seedlings and, presumably, of geotropic hydroids.

That the characteristic position of the stem in *Corymorpha* is not due to a difference in the specific gravities of the distal and proximal regions is apparent when it is seen that not only is the hydroid both proximally and distally heavier than water, but distally it is heavier than it is proximally. If a hydroid is placed in a jar of water after having been slipped out of its proximal investment of perisarc, weighted down as that is by sand clinging to the filaments of the hold-fast, it sinks at once, hydranth first, and lies upon the bottom until the proximal end becomes attached. When this occurs, the stem begins to rise, and in an hour is erect.

The result is in the end the same whether the hydranth is present or absent, whether the stem is cut so that the proximal



portion is two-thirds, one-third, or even one-eighth the original length of the stem. Evidently the stem is generally responsive to the geotropic stimulus. The only difference lies in the time consumed in reaching the vertical position. The longer the stem the shorter the period.

The result is also in the end the same whether the hydroid is hung vertically upside down, by the proximal extremity, or right side up, by the "neck," just below the hydranth, or by the middle. And it matters not whether the hydranth and foot are both or either one present or absent.

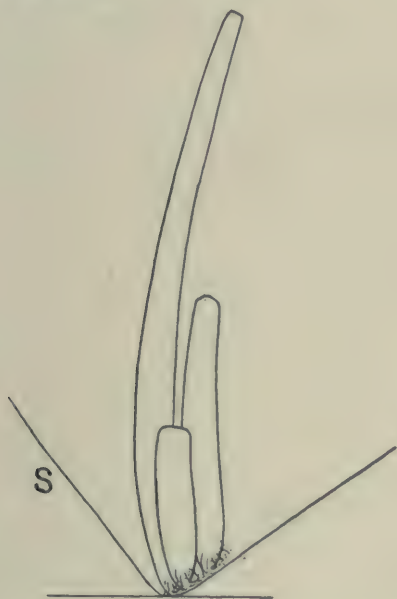


FIG. 1.

Three vertical stems cut at different levels, which were parallel with *S* one hour before.

Numerous experiments justify this summary. Typical cases will be described. To exclude the possible influence of light and oxygen on the direction of orientation, the hydroids were compelled to orient themselves in sealed jars quite full of water, which were placed in dark closets. Check experiments in the light and in open aquaria gave identical results. Further, about a dozen individuals were subjected for three hours to light coming from but one direction, without any observable result on their orientation.



Several uninjured hydroids and three (Fig. 1) which had been cut respectively two-thirds, one-third, and one-eighth the length of the stem from the proximal end, all weighted with sand as usual, were placed in a dark closet in a jar full of water. As soon as they became erect, the jar was tilted at an angle of forty-five degrees, the stems of the hydroids remaining parallel with its sides. In an hour all the stems had become erect. The distal pieces cut from the three mutilated stems were still lying on the bottom, unable to rise for lack of hold-fasts. The jar was brought back to the vertical, and in another hour the stems had swung through forty-five degrees to the vertical, in the opposite direction. The time required for such changes varies considerably, but in the same experiment the shorter the piece the longer the time—ten to twenty minutes longer in the case described. The movement was constantly toward, never away from, the vertical position finally assumed, and did not suggest in the slightest the method of trial and error (Jennings, '04).

The time required for an inverted hydroid to right itself is much longer. Two hydroids, hung vertically by a string tied to their proximal ends, were horizontal within seven hours, inclined upward at an angle of forty-five degrees in thirty hours, and vertical in their normal position in forty-eight hours. In another experiment the hydranths and hold-fasts were removed from two hydroids which were hung on a thread piercing them near their proximal ends. They righted themselves in twenty-four hours. When hung from strings around their necks, the stems remained as they were, vertical, whether they possessed hydranths or not. A stem lacking both hydranth and hold-fast was pierced through the middle by a glass needle which was suspended horizontally. In an hour the stem was vertical, distal end up, and remained thus for several days.

Fig. 1 shows fairly well the important fact that it is unmistakable in the animals themselves, that the stem in turning toward the vertical does not bend locally but generally. *Corymorpha* resembles the stems of plant seedlings in this respect, as well as in the preliminary bending of the stem beyond the vertical. Whether the bending travels progressively from oral to aboral end

was not determined. There would seem to be more than an accidental association in these widely separated organisms of similar phenomena with similar structures.

Another experiment demonstrated that a long exposure in an inverted position to the influence of gravity has no effect on the response of the individual when returned to normal conditions. Two hydroids were suspended vertically upside down in glass tubes, to prevent them from righting themselves. At the end of a week they were freed, and oriented themselves normally.

There are two elements in the stem to which the foregoing results might be referable: the muscles, and the axial endoderm. To solve the problem which thus presented itself the following typical experiments were performed.

A hydroid was decapitated and three wounds made at moderate intervals half through the stem on one side. The stem bent toward the opposite side, showing the greater potency of the unharmed muscles. When, however, it was laid upon the bottom of the aquarium, wounded side uppermost, it assumed an erect position in about an hour. It moved toward the muscularly weaker side as rapidly as it would have done it had the stem been intact. In whatever relation to the bottom the wounds were placed, the stem regained a vertical position in about the same time—in all cases very gradually. Another individual was cut in a similar manner, though in this case there were eight or nine cuts alternately on one side and the other. These cuts interrupted the continuity of *all* the muscles except for very short distances on the stem, which lay quite limp on the floor of the aquarium immediately after the operation. Within two hours, however, it had stiffened into an erect posture. The wounds in these cases did not close for many hours after the stem had become erect.

Only the continuity of the longitudinal muscles was broken by the wounds, whose edges were drawn apart by the contracting muscles. The axial cells not only maintained a continuous column, but bulged out into the gaping wounds in the wall, under considerable internal pressure. Since a stem mutilated on one side may right itself when it is much contracted, and in this condition the muscles as a whole on the wounded side are weaker than

those on the other side, it seems highly probable that the orientation is not the result of muscular activity, but must be due to changes in relative volume of the vacuolated endoderm cells on opposite sides of the stem. And since these cells are exceedingly large, with excessively thin walls and almost no protoplasm, the changes in volume appear to be due to changes in the turgidity of the cells. This conclusion is borne out by the facts that the stem may not only shorten without increasing its diameter, but may lengthen while actually increasing its diameter, results possible only through a variation in the turgidity of the axial cells. A complete demonstration that muscles do not take part in the geotropic response is lacking, because in spite of the numerous transverse cuts made in the stem, the latter was still able to shorten (thickening at the same time), showing that the muscles were not rendered entirely impotent. But the slowness of the response and its occurrence while the wounds gaped and the muscles on the upper side of the stem were manifestly weaker than those on the lower side, strongly support the view that they were not concerned in the result. I think we may say that the muscles produce the movements of the tentacles, proboscis, and all save the geotropic movements of the stem, including shortening and possibly lengthening (by means of the circular muscles) while the axial cells cause the geotropic orientation as well as lengthening of the stem.<sup>1</sup>

If organic growth is increase in volume,<sup>2</sup> then the changes in turgidity which affect the orientation and length of the stem must be reckoned among growth processes, and as such they will be found to differ in no fundamental respect from those growth processes in plants and in all probability the fixed hydroids also which accomplish the orientation of these organisms with reference to gravity. This statement requires some comment.

A comparison of the phenomena of geotropism in the stems of plant seedlings and *Corymorpha* brings out points both of resemblance and difference. The cells reacting to the geotropic stimu-

<sup>1</sup>The skeletal function of the axial cells, correlated with the absence of a supporting perisarc, will be considered in a subsequent paper.

<sup>2</sup>Davenport, '97.



lus are in both cases strikingly similar in structure, being large, with a relatively small amount of protoplasm and large vacuole, and they bring about the bending of the stem by changing their volume. In the seedling, the cells on all sides of the stem increase in volume, but in those on the lower side the increase is greater than in the cells on the upper side. In *Corymorpha*, a similar differential between the upper and lower cells is established, though this may not involve a change in volume of cells on both sides of the stem. Just how it is established cannot be determined at present, for reasons which bring about an interesting but not fundamental difference between the responses in plants and *Corymorpha*. The increase in volume of the plant cells—their growth—is permanent, because it includes growth of skeletal cell walls which prevent the return of the cells to their previous size, and it can be readily measured. The cells of *Corymorpha* have no such walls, and can change their size without difficulty; their growth is temporary and cannot be measured in the same way, because the stem is liable to frequent non-geotropic changes in length. The presence or absence of skeletal cell walls determines whether the growth is to be permanent or transitory. This consideration leads to the discussion of the geotropisms of such fixed hydroids as the sertularians, some of which are known to be geotropic, and all of which are provided with stout perisarcal skeletons.

The experiments of Driesch ('92) on species of *Sertularella*, brought out the facts that the geotropic bending of the stem is not general, but localized in the growing region at the end of the stem, and that the growth which accompanies the bending is permanent. My observations ('02) of *Sertularia furcata* and *S. argentea* in nature are in harmony with these results. "The San Francisco colonies [of *S. furcata*] were growing on erect stalks of *Phyllospadix*. The stems are short and project from all sides of the eel-grass. Each stem leaves the eel-grass at an angle of about thirty degrees, then bends quickly away so that for the most part it makes an angle of seventy degrees with the stalk. The hydrothecæ of the first, and often of the second pair as well, are not in contact. Those of succeeding distal pairs are not only in contact



for half their length but tend much more strongly toward the upper side of the stem than do the proximal hydrothecæ. This would seem to be an instance of the effect of gravity upon the direction of hydranth buds. The farther the stems diverge from the vertical, the more closely do the hydrothecæ of each pair crowd each other on the upper side of the stem" (p. 66). "The habit of the San Francisco colonies of *S. argentea* seems to be controlled in an interesting fashion by gravity. The branches are borne on all sides of the stems, which were fastened by their bases to the perpendicular side of a shore boulder. Each stem had curved upward, so that while the basal portion was nearly horizontal, the terminal fourth or fifth was approximately vertical. In this terminal vertical portion the branches and the hydrothecæ on them were arranged symmetrically with respect to the axis of the colony; and in this region the axis of the colony and the lines of force of gravity were parallel. At the base, where they were not parallel, branches and hydrothecæ were oriented with respect to the force of gravity alone. Both hydranth and branch buds, as well as the stem, thus appear to be more or less negatively geotropic, the hydranths always being borne on the upper sides of the branches; the latter grow away from the center of the earth but never become parallel with the main stem" (p. 68).

It is not difficult to explain why the geotropic bending is at the end of the stem. It is only at the growing tips of stems and stolons that the perisarc is dissolved. Elsewhere the cells of the cœnosarc may contribute perisarc, but are ordinarily unable to dissolve it. At the tip, then, either by means of muscular activity (*cf.* the free *Cerianthus*) or, more probably, by growth processes similar to those taking place in *Corymorpha*—it is not yet determined which—the stem assumes an orientation which is temporary at first, becoming permanent only when the hardening of the perisarc about it prevents further bending.

Enough has been said to show that no fundamental distinctions can be made between the geotropism of permanently fixed, temporarily fixed and permanently free organisms, such as plants, hydroids, anemones, protista. Any theory, then, which seeks to offer a thoroughly satisfactory interpretation of geotropism in one

of these organisms, must be similarly satisfactory for all. The following characteristics of the geotropism of *Corymorpha* appear to find no adequate explanation in existing theories.

Suppose a *Corymorpha* stem (Fig. 2) to be cut at  $x$  into two segments,  $A$  and  $B$ . Now, at whatever point  $A$  be supported above the proximal end, the latter will seek the center of the earth. On the other hand, at whatever point  $B$  be supported, its distal

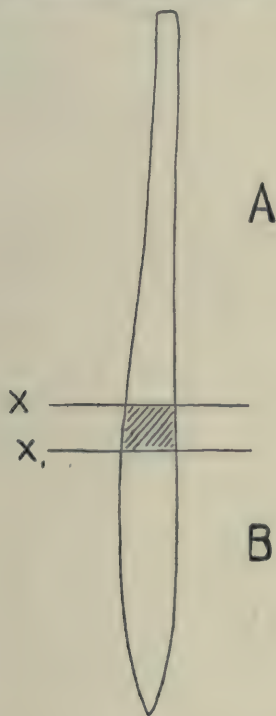


FIG. 2.

Diagram of stem, to illustrate geotropism.

end (cut at the same level as the proximal end of  $A$ ) is strongly negatively geotropic. If the cut were made at  $x_1$ , the shaded portion which, as a part of  $B$  would have been negatively geotropic, would now appear to be positively geotropic. I say "appear to be," for while the negative response is due to a change in the turgidity of the axial cells, the positive response may not be truly geotropic, but may mark an unresponsive period in the axial

cells, induced by a suspension of the stem from its distal end, in which case the stem would come to a vertical position of its own weight. This statement may be nearer the facts and yet not lead us appreciably nearer an explanation. Why should the same cell react in one way when the stem is attached proximally, and another way or not at all when the stem is attached distally?

In seeking an answer for this question, it should be observed that gravity may conceivably stimulate the stem in several ways: (1) through the difference in the mechanical stresses on the two

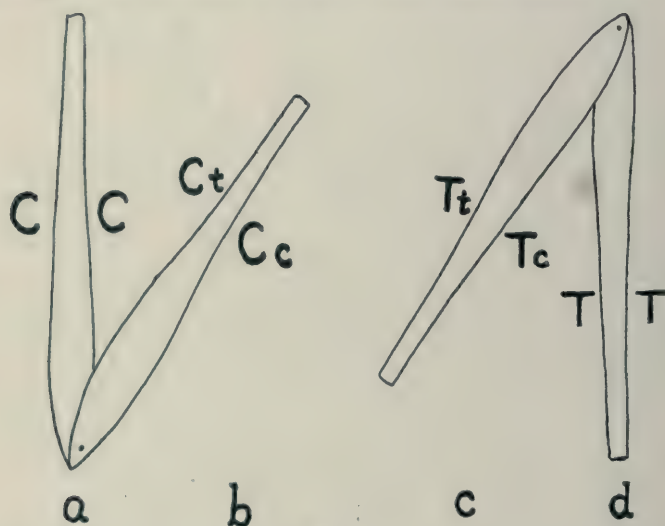


FIG. 3.

Diagram to illustrate geotropism. Cc, compression, greater and less; Tt, tension, greater and less.

sides of the stem, (2) through the difference in the resistance encountered by the organism according as it goes upward (friction + weight) or downward (friction - weight)—Davenport's theory as applied to free organisms, (3) by redistributing the contents of the axial cells so that in any but a vertical position of the stem the cells would be in a state of unstable equilibrium with respect to the geotropic function.

With reference to the first hypothesis, it may seem that the difference in the response of the same cell may depend upon cer-

tain differences in the stresses when the stem is suspended from one end or the other (Fig. 3). If we assume the axis to be rigid to some extent, then, when the stem is anchored proximally ( $a$  and  $b$ ) its weight may tend to compress its elements in the direction of its axis when it is vertical ( $a, C$ ); when it is not vertical ( $b$ ), there may be added to this compression a tension of the elements on the upper side of the stem ( $Ct$ ), and an increased compression of those on the lower side ( $Cc$ ). When the stem is hung from its distal end ( $c$  and  $d$ ), a tension may take the place of the compression ( $d, T$ ), and if the stem be not vertical ( $c$ ), the tension may be increased on its upper side ( $Tt$ ), a degree of compression added to the tension on its lower side ( $Tc$ ). There may be, then, a degree of tension on the upper side and a degree of compression on the lower side of each stem; in which case the differences would be differences of degree only. That differences of degree do not modify the reactions of the axial cells is evident when it is remembered that a stem hung vertically from its proximal end begins to right itself when, according to the hypothesis, the tension factor is strong on both sides of the stem, and continues in the same direction after it has passed the horizontal, *i. e.*, after the tension has ceased on the lower side and become much reduced on the upper side.

The inadequacy of the first hypothesis may be shown further, in the discussion of the second. This view was formulated to explain the orientation of free organisms only. It assumes that negatively geotropic organisms tend to move in the direction of greater resistance; being heavier than water, they would meet with greater resistance in going upward than in going downward. "Another stimulus," says Davenport, "which is probably associated with this, depends upon the fact that an unsymmetrical body, heavier than water, tends to fall with its larger end down." That this view cannot explain the phenomena of orientation in *Corymorpha* will be clear from the following considerations: First, it presupposes locomotion, while locomotion is not concerned in the orientation of *Corymorpha*. Second, if the stem moves in the direction of greater resistance, a stem hung from its distal end ought to move in the same direction as a stem hung from



its proximal end and parallel with the first (Fig. 4). Instead of moving in the same direction, however, they move away from each other as indicated by the arrows, *A* in the direction of *less*, *B* of *greater*, resistance.

It is clear that the factor of external resistance does not govern such behavior, nor does the mechanical factor of tension, as has been shown above. It is equally difficult to explain the geotropic reactions of *Corymorpha* on obviously mechanical grounds by means of the third hypothesis; for the response of a given cell may be different, according as the stem is hung by its proximal or distal end, though the contents of the cell be distributed by

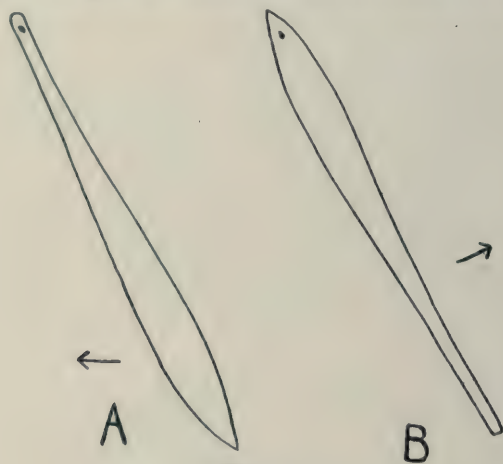


FIG. 4.

Diagram to illustrate geotropism.

gravity in the same way in the two cases. This hostility to the familiar mechanical explanations which appear to account for the facts in other geotropic organisms urges upon me the desirability of repeating and extending my experiments as soon as opportunity is afforded. It is certain, however, that the stem *as a whole* orients itself negatively to gravity, without regard to the point at which it is supported. And the reactions of the axial cells are unquestionably associated with the polarity of the stem. A change in the polarity of a region of the latter is always accompanied by a change in the reactions of the axial cells in this region.

For instance, the cells in  $x-x_1$  (Fig. 2), if forming part of the piece *A*, would as a whole be positively geotropic so long as the aboral end of *A* tended to develop a hold-fast (thus preserving the original polarity of the piece). If, however, this end should develop a hydranth, the behavior of these cells would be reversed; they would exhibit, as a whole, negative geotropism.

There are, then, two manifestations of polarity of apparently different sorts: first, polarity expressed through a special mechanism involving a single tissue, in terms of osmotic pressure and consequent movements of geotropic orientation; second, polarity expressed through a mechanism involving many tissues, in terms of regenerative development and differentiation. However different these may seem, they are undoubtedly referable to the same fundamental causes—the causes of polarity in general, which involve internal factors at present objects of speculation only. Yet it may be possible to determine these internal factors more easily by means of the facts of what may be called functional polarity than by the relatively complex morphological phenomena of development and differentiation. The simpler mechanism and the simpler effects of polarity as manifested in the axial cells, are bound to bring a true explanation of polarity nearer our comprehension, although it may still be unattainable. How an organic membrane or its contents may change in order to produce a change in osmotic pressure, while an enormously difficult problem, is yet more hopeful of solution than the problem of how several tissues simultaneously differentiate in different directions to produce a complicated regeneration.

### *c. Locomotion; Amœboid Cells.*

We come now to a third type of movement, with a new cause. The ectoderm cells of the proximal end of the stem are capable of amœboid movements, by the aid of which the hydroid may slowly change its location. In this regard *Corymorpha* closely resembles *Hydra*. On a horizontal surface, whatever locomotion there is takes place in any direction, with the one qualification that the stem moves always out of its perisarcial investment, which it leaves behind.

The rate of locomotion is slow. Half an inch in twenty-four hours is a maximum rate. On a vertical surface the movement is always directly upward. Gravity evidently determines the direction.

The value of locomotion of this sort, and especially its negatively geotropic character, would seem to lie in providing a means whereby the hydroid may keep above the surface of the shifting sands.

The filaments of the hold-fast are also furnished with amœboid cells by which they are enabled to move out amongst the sand grains to which they cling and anchor the stem (*cf.* amœboid movements of the tips of stolons of Campanularian hydroids). One set of observations gave a rate of nine microns per minute at the tip and five microns per minute half way to the base of a filament several millimeters long. The free end is swollen and club-shaped, with well developed ectoderm which not only provides amœboid cells but gland cells, which secrete the perisarc in which the final strength of the filament as an anchor lies. The ectoderm of the remainder of each filament is attenuated almost to the limit of visibility; the whole filament appears to be upon the stretch, pulled out by the creeping club-shaped end. The endoderm of the filament is composed of a single column of cells such as is characteristic of the endoderm of the tentacles of Campanularian hydroids. These cells, under the tension, may become much longer than broad, and retain these proportions whether the filament is attached or free. A "setting" process seems to have followed the stretching here, effecting the permanence of the attenuation without cell division.

The direction of locomotion of the filaments is always *outward*, but appears to be otherwise indeterminate. Arising below the perisarc on the peripheral canals of the stem as solid outgrowths with a deflection toward the proximal end, they creep along the stem for a short distance, closely in contact after the manner of stolons, and then push outward, secreting perisarc as they go. If the stem is hung freely in the water, the filaments extend in all directions. If it is in contact with the substratum, however, they creep along the latter as soon as they come in contact with it. If the substratum is sand, a filament pushes its way between the



grains, as a plant root pushes its way through the earth. In no case, however, does it appear to respond to the stimulus of gravity, or any other stimulus, except that of contact. Resistance seems to incite movements which overcome it. This is probably the reason why the filaments leave the easy path between stem and perisarc to push out against the resisting wall of the latter.

#### *d. Circulation; Cilia.*

A fourth type of motion is found in the currents set up in the cavities of the digestive tract by means of cilia. The cilia are borne on the lining cells of the proboscis of the hydroid and the epithelium bounding the peripheral canals on their outer side. They are present throughout the peduncles bearing the medusæ, and the manubria of the latter.

There are variations in the currents, particularly in the peripheral canals. At times there may be no current at all. At others the current may be setting very rapidly in the same direction in all the canals visible. Abrupt reversals occur under these conditions, which can hardly be explained by ciliary action, but are rather the result of expansions or contractions of the proboscis and stem, which produce changes of pressure in the canals.

#### IV. THE YOUNG HYDROID.

The eggs are laid by medusæ which are never set free from the hydroid. They are small but heavy with yolk and fall directly to the bottom in quiet water, adhering by their delicate coats to the first object they touch.

As soon as the egg is attached, its free life is practically over. *The embryo is never ciliated, and has no free-swimming phase in its existence.* It is capable of very slow creeping movements,<sup>1</sup> however, by means of which it often comes in contact with other embryos and forms with them temporary associations of as many as six, ten, twelve individuals. Often it will travel many times its own length, leaving behind a narrow collapsed tube of perisarc

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<sup>1</sup>*Cf. Hypolytus peregrinus*, Murbach (Q. J. M. S., XLII, 1899, p. 341), which it resembles in this and other respects.



which it has secreted and which is continuous with the egg case. As we have seen, the hydroid never loses its power of locomotion, even after the development of the filaments of the hold-fast.

As the embryo leaves its egg case, it elongates, and an anterior (oral) end can be distinguished from the narrower posterior (aboral, proximal) end. The anterior end soon elevates itself, and the embryo now touches the substratum by one side of the aboral region only.

For about thirty hours, or up to the time when the hydranth is beginning to form, the embryo is completely covered by an

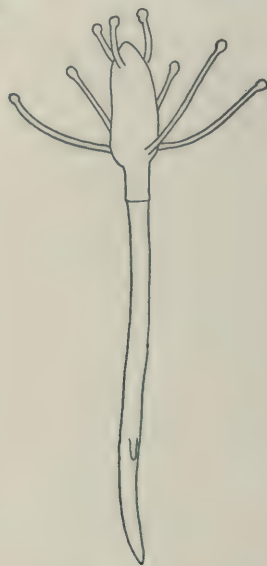


FIG. 5.

Young *Corymorpha*.

extremely delicate layer of perisarc. From this time the perisarc is frequently limited entirely to the stem. Before the formation of the hydranth, the perisarc covering the anterior end of the embryo, and secreted by glandular cells of the ectoderm, is not permanent, being dissolved as the stem progresses, probably by the secretion of other cells in this region. As the hydranth begins to develop, its ectoderm ceases to manufacture perisarc, which henceforth is deposited by cells beginning at the aboral limit of

the hydranth. The perisarc is hardly sturdy enough at any time to afford any support to the stem. Its adhesive character, however, serves to attach a portion of the latter to the substratum, over which the cœnosarc creeps.

Amœboid ectoderm cells are responsible for the locomotion of the young *Corymorpha* (Fig. 5) as of the adult, though they are not confined to the proximal end of the stem. Often the latter clings for half its length and may perform looping movements, much less pronounced, however, than those of *Hydra*.<sup>1</sup> The direction of locomotion is also determined by the same factors which regulate it in the adult. On a horizontal surface the direction is indeterminate, though the stem always moves *out* of its investment. On an oblique surface it tends to move upward by the nearest route. Young hydroids are often found adhering to the stem of an adult, the relation of the axis of the attached portion of each to the adult axis varying with the inclination of the latter. If it is vertical, they are parallel with it, vertical also; and the rest of the young stem will be nearly vertical, but not quite so since the distal portion of the stem seems to shun any contact (negative thigmotropism). If the orientation of the adult is altered, the young hydroid will gradually take up a new position in which the most distal point of attachment will be the greatest possible distance above its proximal end.

Not only, therefore, is the larva negatively geotropic with regard to orientation, but this has a directive effect upon locomotion. It is probably due to the effect of the stimulus of gravity on the endoderm cells which line the single cavity and from which the axial cells of the adult are derived. These cells do not contain such enormous vacuoles as those in the axial endoderm, and are ciliated. In these respects they resemble the parietal endoderm cells of the peripheral canals of the adult, which are their descendants also.

With reference to the amœboid cells which produce locomotion in *Corymorpha*, it may be recalled that the ectoderm of hydroids is not uncommonly amœboid. To cite but a single instance, not

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<sup>1</sup>Cf. Marshall. Zeitschr. f. w. Zool., XXXVII, p. 664.

only is the cauline cœnosarc in Campanularian hydroids, *e. g.*, *Obelia*, fastened to the perisarc tube here and there by multicellular amœboid processes of the ectoderm, but the anterior ends of growing stolons exhibit amœboid changes of form which account for their creeping movements and produce the tension often manifested in the cœnosarc which is fixed farther back on the stem. The cœnosarc is literally dragged out of the perisarc. A similar tension has already been noted in the filaments of the hold-fast, due to a similar cause. And it is probable that the proximal end of the larva may be dragged along at times after the more distal attached portion.

The active muscular movements discussed at length for the adult need not be considered here, as the young hydroid appears to respond similarly in all respects, with the one exception that the reaction times are somewhat greater.

The absence of a free-swimming larval form seems to account for the tendency of *Corymorpha palma* to dwell in communities, as previously mentioned. The power of locomotion is too slight to have any effect on the distribution of individuals, which is accomplished by tidal currents and the shifting of surface sands. Occasionally an individual may be washed away from its anchorage, and begin a new community in a new locality.

#### SUMMARY.

*Corymorpha* is unusually active for a hydroid. It is everywhere sensitive to mechanical stimuli, irritating chemicals and abrupt changes in temperature, nowhere to "odorous" substances. The prehensile mechanism is composed of proximal tentacles, which move toward the mouth in response to all effective stimuli; distal tentacles, which move away from the mouth in their initial response to stimuli; and proboscis, which may move toward the point stimulated. These movements, as well as shortening and possibly lengthening the stem, are performed by muscles.

The stem of the adult responds to the stimulus of gravity, by means of a change in the turgidity of the vacuolated axial cells. The response of these cells varies according as the stem is attached

proximally or distally, and according as it is heteromorphic or not. The polarity of the stem is expressed, not only by the regenerative development but by the changes in the axial cells.

Locomotion is accomplished by amœboid cells located at the proximal end in the adult, more generally distributed in the larva, and covering the club-shaped ends of the filaments of the hold-fast.

Cilia are present on the epithelial cells lining the hydranth cavity and peripheral canals. Supplemented by contractions and expansions of the hydranth cavity, they provide for the circulatory currents through the body.

Eggs are laid both in summer and winter, usually during the morning hours. They have adhesive coats. The planulæ are never ciliated, and their locomotion is limited to very slow creeping movements. The larvæ are geotropic.

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# STUDIES ON THE LIFE HISTORY OF PROTOZOA.

## IV. DEATH OF THE A SERIES.

### CONCLUSIONS.

BY

GARY N. CALKINS.

WITH 3 PLATES AND 3 FIGURES IN THE TEXT.

Experiments on the life-history of *Paramœcium caudatum* have now been carried on continuously for 29 months. Two series, designated as the "A series" and the "B series," were started on the first of February, 1901, with individuals from different sources. The B series died out in May, 1902, in the 570th generation; the A series on December 19, 1902, in the 742d generation. A third series—"C" was started in June, 1902, with an individual from Cambridge, Mass., and died out in June, 1903, in the 379th generation. The progress of the first two series has been recorded from time to time,<sup>1</sup> and in the present paper I wish to give the history of the last cycle of the A series and to consider the results in relation to some general biological problems and theories.

#### I. THE JUNE AND DECEMBER (1902) PERIODS OF DEPRESSION.

As described in the earlier Studies (I and III) the general vitality of the two series, A and B, as expressed by the daily division rate, underwent periodic cycles of vigor and depression.

<sup>1</sup>(1) Studies on the Life History of Protozoa. I. The Life Cycle of *Paramœcium caudatum*. Archiv. f. Entwk. XV, 1, 1902.

(2) Studies, etc. II. The Effect of Stimuli on the Life Cycle of *Paramœcium caudatum*. (With C. C. Lieb). Arch. f. Protistenkunde. I, 1, 1902.

(3) Studies, etc. III. The 620th Generation of *Param. caud.* Biol. Bull. III, 5, 1902.

The early curves appeared to indicate a periodicity of three-month intervals, and this was taken to be the time of the usual life cycle in culture of *Paramæcium caudatum*; this conclusion was based partly upon my own results and partly on those of Joukowsky and of Simpson, both of whom found that cultures of this infusorian died out after three months of treatment. It was found, however, and it may be seen from the now completed curve of the A series (see Diagram I) that trimonthly periods of depression were not fatal and that recovery occurred without purposeful stimulation. Thus in the first apparent depression (May, 1901,) the recovery was thought to be due to the stimulation by jolting on a railroad trip of six hours; another in March, 1902, was considered due to a slight rise in temperature. These periods of depression differ markedly from those of August and December, 1901, and of June, 1902, when the individuals continued to die at a high rate, notwithstanding repeated jolting experiments, increase in temperature, and the like, and the race was saved only by change to a special diet after numerous attempts and failures with foods of different kinds. The well-marked cycles, therefore, with periods of depression which demanded stimulation of a decided character, were approximately of *six months'* duration, while intermediate cycles of less importance were about three months long. The first of the six-month cycles ran from February 1, 1901, to August 1, 1901, (see Diagram I); the second from August 15 to January 1, 1902; the third from January 1 to July 1, and the last from July to December 19, 1902. During the first three cycles the number of generations was nearly the same (200, 198 and 193, respectively), the last, on the other hand, was much less, the individuals dividing only 126 times.

The stimulation which resulted in the renewal of vitality after the periods of depression in August and December, 1901, was due to the change from hay infusion diet to beef extract for a limited period (see Studies I and III). The same change failed to work in the July, 1902, period of depression, and after the race had become reduced to only six individuals, a successful substitute for the beef extract was found in the extracts of pancreas and brain (see Studies III). Recovery, however, was not so



successful as in the previous periods and the organisms were much less vigorous than at similar periods in previous recoveries. The division rate, furthermore, slowly fell from the relatively high point in August, and gradually decreased during the fall months until the A series died on the 19th of December. The B series had succumbed in the 570th generation, in June, before the right stimulus was found. Except for the slowness of divisions the organisms appeared perfectly healthy during the summer and fall of 1902, although microscopical study of preparations made during this period showed characteristic changes in the protoplasmic structure (see Figs. 18 to 21). The organisms were plump and moved freely about the slide, responding with customary vigor to stimuli of different kinds. Every precaution was taken during this period to invigorate the race and every experiment that my ingenuity could devise was executed; some appeared to give a temporary improvement but none was permanent, and the last individual of the A series finally died after 23 months of continued daily observation, without, however, any morphological evidence of general senility. (See Diagram I.)

## II. UNSUCCESSFUL ATTEMPTS TO REJUVENATE THE A SERIES IN THE FINAL PERIOD OF DEPRESSION.

Artificial rejuvenation of the A series was successfully accomplished three times. The experiments and results have been described in other places, and it will be sufficient here to merely point out that after considerable experiment, beef extract was successful in the first two cases and pancreas and brain extract in the third, the result being due, probably, to the change in salt contents of the medium. The approaching end of the series was indicated some time in advance by the reduced division rate during the fall of 1902, and efforts were continuously made to rejuvenate them during this period. For these experiments all of the stock of the regular series was maintained, and the number of lines under observation frequently ran up to twelve or more. The results of all experiments were tabulated and the effects of the stimuli used were noted for comparison with the regular series. The general result may be seen upon the diagram which





hows that, despite all efforts to stimulate, the race rapidly weakened and ultimately died.

### 1. *Experiments with Extracts.*

a. *Beef Extract.* During the last autumnal period, beef extract was used at different times in the same way that it had been used successfully during previous periods of depression, and for the same length of time, twenty-four hours. The organisms were immersed in the fluid full strength in the majority of cases, but experiments with the half strength were also made. The failure of the beef treatment in June, 1902, has already been described (Studies III) and I shall consider in this place only the experiments subsequent to that time. In general it may be stated that beginning with the treatment in May, the effect of the beef extract was nil. On the 19th of June A<sub>3</sub> and A<sub>4</sub> were immersed as usual and for the same length of time. Both of them died before the 27th. Again on the 22d A<sub>1</sub> and A<sub>2</sub> were treated, and both of these died on the 26th. Similar results were obtained in all later experiments, as shown by the following résumé:

July 6.	A <sub>3</sub> and A <sub>4</sub> treated.	Both died before the 15th.
July 24.	A <sub>1</sub> , A <sub>2</sub> , A <sub>5</sub> “	A <sub>1</sub> and A <sub>2</sub> died on 25th. A <sub>5</sub> on the 26th.
Aug. 2.	A <sub>1</sub> and A <sub>2</sub> “	Both died before the 19th.
Aug. 2.	A <sub>5</sub> and A <sub>6</sub> “	Both died before the 13th.
Nov. 26.	A <sub>5</sub> “	Died on the 27th.
Nov. 26.	A <sub>7</sub> “	Died on the 29th.
Nov. 26.	A <sub>11</sub> “	Died December 2.
Nov. 28.	A <sub>6</sub> , A <sub>8</sub> , A <sub>9</sub> and A <sub>12</sub> treated.	Died before December 4.

The short time in beef may not have been long enough to make the change beneficial; with this in mind I kept the last few individuals in for three days (Nov. 26, 27, 28). Not one of them divided more than once and all died within a week. Beef extract, therefore, had lost its potency as a rejuvenating medium.

The effect of beef extract upon the body structures was to increase the number of gastric vacuoles; while, in some cases, the micronuclei were caused to divide (Figs. 5 and 6). Even in May, 1902, there was an indication of the endoplasmic concentration

which accompanied depression at this period. The dense condition of the protoplasm is better shown in Fig. 6 which represents an individual twenty-four hours after transference from beef extract into hay infusion. It may be noted here that, at this period, the beef extract failed to reduce the dense endoplasmic condition to one of tenuity which seems to be the normal condition.

b. *Extract of Pancreas.* Extract of pancreas was made in the same way as the beef extract. A fresh sheep's pancreas was cut in small pieces and brought to boiling point in water. After filtering and cooling, the *Paramœcia* were placed in it and left for 24 hours, as in the beef. At first it proved a good substitute for the beef and the organisms appeared to thrive on it; but later, in November and December, it was as useless as the beef. The following records show this fact:

June 27.	A3	treated.	Divided twice in 24 hours. Forms the regular series from this time.
June 29.	A1 and A2	"	Divided a few times. Died out on July 14.
July 16.	A4	"	Died the next day without division.
July 17.	A6	"	Divided twice the next day; lived.
July 18.	A1, A2 and A4	"	All died the next day.
July 20.	A1, A2 and A4	"	Lived. Given <i>mutton broth</i> on 23d. Died on 24th.
Aug. 20.	A2	"	Died on 28th.
Aug. 20.	A8	"	Died on the 21st.
Dec. 8.	A5, A6, A7 and A8	treated.	No divisions. All died out on 19th before or after treatment with various other substances.

At the period in June when recovery was effected by using the extract of pancreas, the organisms of both series were in the condition represented by Fig. 7. The endoplasm was densely granular and homogeneous, and had a curiously "stuffed" appearance. This condition was relieved by using extract of pancreas, whereas beef extract, made with the same water and in the same way, was ineffectual. Figs. 8, 9 and 10 show the general course of the

action of the pancreas extract. Fig. 8 represents an individual twenty-four hours after treatment, *i. e.*, after change from pancreas extract into hay infusion.<sup>1</sup> The characteristic dense structure is distinctly shown, but in the center there is unmistakable evidence of the normal condition. Figs. 9 and 10 represent two individuals forty-eight hours after treatment with the pancreas extract. In the former, the characteristic dense structure is still visible at the two ends, but the center is clearing. In the latter, new gastric vacuoles have appeared in the endoplasm, the animal being well on toward recovery when killed.

c. *Extract of Sheep's Brain.* This was made in the same way as the other meat extracts, and the animals were similarly treated with it. It was not efficient as a permanent stimulant, and was discarded in subsequent treatment.

d. *Extract of Mutton.* "*Mutton Broth.*" This extract was also tried in the summer (July 20 and 23), but in no case was it successful, the organisms invariably dying within 24 hours.

e. *Lecithin.* A trace of pure lecithin was put into the regular hay infusion during the week of August 20. The organisms were apparently not injured by the change, but did not live more than 48 hours after the treatment.

f. *Pineapple Extract.* With the view of ascertaining if some of the vegetable ferments might not prove beneficial, I tried extract (juice) of fresh pineapple, and of fresh apple. A4 was put into dilute pineapple juice July 27. The reaction was well marked, as shown by decided increase of movement and by three divisions in the ensuing 48 hours. The experiment was repeated the next day with a like result. It was repeated again August 3, but was unsuccessful, the organisms dying two days after treatment. The stimulation was temporary in all cases, and it should be noted that the organisms were in a period of increasing vitality when the first pineapple treatment was given (see Diagram I).

<sup>1</sup> The hay infusion was made every day, the same amount of hay and water being taken each time and raised to the boiling point. This method was never varied during the entire period of the cultures and the salt content of the water, as shown by weekly analyses, did not vary beyond a very slight fraction of one part to one hundred thousand.



g. *Apple Juice.* A piece of fresh Porter apple was allowed to lie for a few minutes in the hay infusion. In this case the result was well marked, and a decided stimulus was noted. Again, on Sept. 20, A5, A6, A7 and A8 were all put into one drop of apple juice to 12 drops of hay infusion and left for thirty to forty-five minutes. They were then transferred to clear hay infusion and left. All divided the next day. The experiment was repeated on the 21st with a like result. In some cases the organisms died immediately, showing that the strength used was too great. When properly diluted, however, apple seemed to give a satisfactory temporary stimulus, although in no case did the stimulation last for more than forty-eight hours. The same experiment tried in October gave no results; the organisms died.

In addition to the above, various proprietary mixtures were tried from time to time. Among these were phospho-albumin, and nuclein-albumin; none gave satisfactory results.

## 2. *Experiments with Acids and Salts.*

In view of the successful results which have followed experiments with ions in connection with egg development, it was thought that perhaps dilute acids or salts would have a beneficial result in the case of these weakened infusoria. Normal solutions were made in each case and various strengths were tested from those that would kill to those that only slightly stimulated. The organisms were left in the fluids for only a short time (20 to 30 minutes) and were then transferred to fresh hay infusion. Attention may be called here to the fact that potassium phosphate when used in this way was successful in restoring the vitality of weakened *Paramæcium* in the preceding December cycle, the "rejuvenation" which resulted was directly comparable with that effected by the beef extract. There was reason, therefore, to believe that the repeated use of various salts would give satisfactory results in the last period of weakness of the race. This expectation, however, was not realized for none of the chemicals used in the fall and winter of 1902 was successful in this way; all were as futile as the beef and pancreas extract, as shown by the following experiments:

## A. Potassium Salts.

a.  $K_2HPO_4$ . On the 8th of June, 1902, one individual from the line of A2 was treated for 30 minutes with a solution of dibasic phosphate of the strength of one drop of  $\frac{n}{1250}$  to six drops of usual hay infusion. The result was a marked increase in the rate of division for a considerable period as compared with the control series, as follows:

Average daily division-rate for 5-day periods, June 8–July 5.

	Stimulated A <sub>2</sub> .	Control Series.
1st Period.....	.8 .....	.8
2d " .....	.8 .....	.6
3d " .....	.8 .....	.6
4th " .....	1.0 .....	.2
5th " .....	1.6 .....	.0

On the 27th of June the above experimental series was substituted in the regular culture series and the descendants of these individuals formed the regular lines until the final extinction, subject, of course, to the other experiments as stated elsewhere.

A stronger solution (1–5) and for a longer period (1 hour) was used with A3 on June 26. The individual died in three hours. On August 21 the same strength was used, but for only 25 minutes; the individual died in four days without dividing. The general effect of this salt was, therefore, favorable, with evidence that a certain optimum strength is alone beneficial. The beneficial effects upon the endoplasmic structure are shown in Figs. 11 and 13.

b.  $KH_2PO_4$ . Experiments with the monobasic salt were also made and various proportions were used, but none was successful.

c. KCN. Various proportions of  $\frac{n}{50}$  of this salt were used, the most successful being one drop of the solution to twelve drops of hay infusion. This was not strong enough to kill the bacteria which afford the only food for *Paramæcium*. Four individuals were immersed October 29 in the mixture and left for 24 hours. At the end of this time each had divided once, while none of the control series had divided. Of the eight individuals resulting from this treatment, four were placed again in the KCN solution,

(made fresh), while the other four were placed in hay infusion without the salt. Of the former set, each individual divided once in 48 hours, and of the latter set, one died, two divided once, and one divided twice in the same period. The regular control series did not divide at all during this time. Both sets were placed in hay infusion on the fourth day and neither set continued to live, all died before the sixth of November. Another set of four individuals were treated with the salt every day for the same period. After the first 24 hours none had divided; after the second 24 hours each one had divided once. This was November 2. On the 4th all had divided twice, on the 6th only one had divided again, on the 7th another one had divided, on the 8th none had divided again, on the 9th one died, while the rest did not divide, on the 10th two others died, and the remaining one was placed in the usual hay infusion without the salt, having been treated daily for ten days with it. It did not divide again until the 18th, and finally died out on the 21st. Others, however, that had been treated, and had been placed earlier in hay, continued to live and supplied the regular lines of the experiment. The use of the KCN therefore can be said to have been successful to a limited extent, and, possibly, to have prevented an earlier extinction of the race. The effect on the curve of the life cycle is shown by the temporary rise during the last period in October and the first period in November.

*d. KOH.* This was tried only once with four individuals on the 28th of October.  $\frac{1}{400}$  I part to 4 was used for 30 minutes. On the first of December two had died and one had divided once. None divided again and all of the individuals experimented with died before the fourth of November.

## B. Sodium Salts.

*a. Dibasic Sodium Phosphate.* Three individuals, A<sub>1</sub>, A<sub>3</sub> and A<sub>4</sub> were placed for 30 minutes in  $\frac{1}{250}$  Na<sub>2</sub>HPO<sub>4</sub>. All died without dividing by the 27th. The experiment was not repeated.

*b. Sodium Tartrate.* July 10 two individuals were placed for 30 minutes in  $\frac{1}{30}$  sodium tartrate, one drop to five of hay infusion.



They died in twenty-four hours. The experiment was repeated on July 14, three drops to five of hay infusion being used. On the 15th they did not divide, on the 17th they divided once, and died on the 18th. Experiment not repeated.

*c. Sodium Chloride.* This salt was used on several occasions with negative results as a rule. (See, however, table below.) In September, 1902, when the race was comparatively vigorous, an individual was treated for 30 minutes with  $\frac{n}{30}$  NaCl, one drop of the salt to twelve of the hay infusion. At the end of 48 hours it had divided once, but died within five days without further division. The effect upon the protoplasmic structure was not particularly noticeable (see Fig. 18).

The following table gives a comparative view of the efficiency of different salts on the division rate for thirty days subsequent to treatment. Several individuals of the A series were treated on the 20th of March with potassium phosphate and the progeny of one of these in the 78th generation were again treated in part on May 6 with potassium phosphate, and in part, with potassium chloride, magnesium chloride, sodium chloride and calcium chloride with the strengths, and for the times indicated. The following notes were made at the time of the treatment. "When the individual was put into the potassium chloride it began at once to swim backward with great rapidity, and continued this for about five minutes. It then straightened out and appeared perfectly normal in the solution. When returned to the hay infusion at the end of the treatment, it went through the same convulsions but soon became normal, perhaps slightly swollen and transparent." Again: "When the individual (another individual of course) was put into the magnesium chloride solution it was hardly affected in any way, a very slight increase in movement being noticed." Again: "Treatment with NaCl did not affect the individual, it appears fat and happy in the hay-infusion." Again: "Very much affected by the  $\text{CaCl}_2$  solution. One of the three specimens died; the other two were distorted and badly shrunken, this lasted for at least fifteen minutes after they had been transferred to the hay infusion."



Average number of divisions per day after stimulation.

	K <sub>2</sub> HPO <sub>4</sub> .	KCl.	MgCl <sub>2</sub> .	CaCl <sub>2</sub> .	NaCl.
May 6-10.....	1.60	1.00	1.00	0.60	1.20
May 11-15.....	1.00	1.20	0.60	1.20	1.20
May 16-21.....	1.50	1.50	1.00	1.50	1.66
May 22-26.....	1.80	2.00	1.80	1.80	2.00
May 27-June 1....	1.25	1.03	1.25	0.75	1.50
June 2-6.....	1.33	1.33	1.33	0.16	1.33
June 7-12 .....	0.20	0.40	1.60	dead	0.40
	dead	dead	living		dead

All were normal solutions, diluted 25 times, one drop to twelve drops of hay infusion and the treatment lasted for 30 minutes in case of KCl, and for 25 minutes in each of the other solutions. Definite conclusions cannot be drawn from one set of comparisons for it may have been pure accident that the magnesium chloride specimens continued to live. The effect of MgCl<sub>2</sub> upon the protoplasmic structures is shown in Fig. 12.

Comparatively few experiments were made with acids. Hydrochloric and nitric acids were tried during the period of depression in October, 1902, but the results were negative, the individuals dying within twenty-four to forty-eight hours. An interesting effect was produced by treatment with dilute phosphoric acid. The dense endoplasm was broken up and with it the macronucleus which, after the treatment, appeared as many small fragments (see Fig. 16).

Of the other unsuccessful attempts to rejuvenate the race during the last period of depression I will mention only those with galvanic stimuli, with nitro-glycerine, and with dried and powdered *Paramœcium* of an entirely foreign race.

### 3. Galvanic Stimuli.

A small cell was made and connected with two Mesco batteries. Four individuals were treated on November 28, three different times to the full current and for a period of one minute each time. The usual reaction followed the treatment, migration to the negative pole, and when the current was reversed, migration from the

positive to the negative pole. At the end of the treatments the four individuals appeared normal. On the following day one had died, another on the ensuing day, and the last two on the fourth day. Another time the same experiment was tried but with only one minute of exposure. The result was the same, death without division. The death of these organisms at this time cannot necessarily be ascribed to the treatment, for a glance at the diagram shows that the entire race was dying and that divisions were infrequent in all cases.

#### 4. Nitro-Glycerine.

At the suggestion of Professor Wilson, and as a last resort, I tried two experiments when the race appeared to be dying out in December. Nitro-glycerine in very weak solution (unfortunately I have no record of the strength used) was put into the hay infusion. It made no appreciable difference in the final result and the organisms did not divide.

Professor Wilson's other suggestion seemed more hopeful, on the *a priori* ground that renewal of vitality is effected by the union of two individuals. A culture of *Paramæcium* fresh from pond water was made, and hundreds of individuals were allowed to dry in a small drop of water in a watch crystal. When dried the remains were scraped together and pulverized, the powder thus formed being added to the hay infusion in which the weakened *Paramæcium* were kept. Although this extremely ingenious suggestion was worthy of a fruitful result, the outcome of the experiments was the same as with all the rest, and not a single individual lived after the 19th of December, one week after six-day treatment with the dried *Paramæcium*.

There remain many experiments that might have been tried, and that might possibly have accomplished the same results that were obtained in the earlier periods of depression when the race was successfully reinvigorated by artificial means, and even the experiments that were tried might have been successful if different strengths, or times of action, had been used. Many suggestions were made by my colleagues and other friends, especially in regard to the trial of some chemical compound. I am pleased to

acknowledge the friendly and scientific interest which prompted these suggestions, and desire to state that if they were not always carried out, it was because of the limits of my time and of the constantly decreasing number of individuals left to experiment with. It was my desire to try as many classes of experiments as possible, and some of these might have been successful if tried at an earlier time or if carried out on a sufficiently large scale, but here again the scarcity of living material would not allow the continued experimentation along lines that were fruitless on the first trial. It must be remembered that such experiments, to be of any value in a work like this, had to be made on the material that had been under constant observation for nearly two years, and preliminary experiments with fresh forms from the ponds were valueless so far as indicating the effect on the vitality of the race under observation.

### III. PROTOPLASMIC STRUCTURES OF PARAMÆCIUM.

#### 1. *The Normal Paramæcium.*

The usual size of a normal *Paramæcium* is from 200 to 300 microns, and the form is fairly constant, warranting the designation "slipper animal." In all of the preserved specimens that I have made from time to time, the fixing fluid was saturated corrosive sublimate to which was added 10 per cent of glacial acetic acid. Having a common method of fixation the different individuals can be compared point by point.

*a. The Endoplasm.* The endoplasm of a normal form is made up of various granules of different sizes, of vacuoles and crystals (Fig. 1). When the animal is moving about in a nutrient medium it constantly takes in food with the water absorbed. The food of *Paramæcium* consists of bacteria, and these accumulate in a gastric vacuole until the latter has attained a certain size when, according to Wallengren,<sup>1</sup> it is caught up in the endoplasmic flow and carried to the posterior end of the body. It then moves anteriorly toward the left side, ultimately passing over to the right and then down on the right side. In this migration of

<sup>1</sup> H. Wallengren. Inanitionserscheinungen der Zelle. Zeit. f. Allg. Physiologie I, 1, 1901.



the vacuole the food is brought into the immediate vicinity of the macronucleus where the effect of the nuclear environment is shown by the immediate acid reactions with congo-red of the vacuole contents (Wallengren). The food material in such a vacuole is massed into a more or less homogeneous body corresponding to Greenwood's observation on *Carchesium*, and in this condition the digestive fluids work upon it to resolve it into digestible and indigestible parts. After this the soluble portions are absorbed and the residue defecated. The soluble portions pass into the endoplasm to be stored up as reserve food (Wallengren) from which they are taken as the need comes to be made into living molecules.

The processes of digestion thus given rise to definite elements in the endoplasm, elements which react to stains in characteristic ways. In addition to these, however, we might expect to find waste matters due to incomplete oxidation as well as final products of metabolism in the form of crystals, etc. The various possibilities of this nature have given rise to different interpretations upon which my own observations throw but little additional light. With neutral-red acting upon the organism when alive, Prowazek<sup>1</sup> distinguished three kinds of granules in the endoplasm: (1) The food balls; (2) Small round granules which are distributed throughout the periphery more or less uniformly (Prowazek actually found them at the two extremities and about the mouth); (3) Minute granules distributed throughout the endoplasm and all over the body.

The minute peripheral granules (No. 2) are interpreted by Prowazek in the same way that Wallengren had previously interpreted similar bodies in *Pleurocotes hydractiniæ*, viz., as excretory vacuoles with a solidified granule of excreta within them. Pütter,<sup>2</sup> on the other hand, interpreted them as basal bodies of centrosome nature lying at the bases of cilia. Wallengren subsequently showed, however, that the granules in question are not at the bases of cilia but lie beside the cilia, and that rows of these granules alternate with rows of cilia. He interpreted them as the

<sup>1</sup> Vitalsfärbung mit Neutralrot an Protozoen. Z. wiss. Zool. Bd. 63, 1898.

<sup>2</sup> Studien über Thigmotaxis bei Protisten. Arch. f. Anat. u. Phys. 1900.



papilliform external swellings of the trichocysts and as merely condensed peripheral portions of the cortical plasm. My own observations support those of Wallengren.

The third type of granule is interpreted by Prowazek as a ferment or enzyme bearer. Pütter, on the other hand, believes them to be "respiratory granules" owing their staining capacity to the contained carbon dioxid. Wallengren's observations on starving forms led him to the belief that neither interpretation is correct, for, he argued, these granules being the first to disappear in hungry forms must be of the nature of stored food (see Figs. 23 and 24).

The crystals which are found in well-fed forms were identified by Schewiakoff as metaphosphate of calcium. They are of various forms and sizes and are confined to the endoplasm; being crystalline in nature they cannot be mistaken. They are now generally regarded as late metabolic products resulting from proteid digestion.

*b. The Ectoplasm.* As in the majority of holo- and heterotrichida the ectoplasmic modifications are well differentiated from the endoplasm. A cuticle and underlying cortical plasm may be made out, the latter consisting of a much more dense substance than the endoplasm, analogous, probably, to the ectoplasm of an amœba. In it are embedded the characteristic trichocysts which ordinarily project ever so slightly from the surface, giving rise to the minute papillæ which may be distinguished in profile between the furrows of the cilia (shown in Fig. 20). In *Paramœcium* taken fresh from the pond water, the fixing agent which I have used, preserves the trichocysts within the cortical plasm, but after a few months under cultivation these organs cannot be made out, and seem to have been discharged and lost under the stimulation of the fixing fluid. Wallengren believes that they are taken into the endoplasm and digested as food in starving forms, but in preparations made from my cultures they are absent in the well-fed forms as in the degenerate ones. In all cases the spaces that were occupied by the trichocysts are present in the cortical plasm as vacuoles, and it is in this state that the relation to the peripheral papillæ can be easily made out (*cf.* Figs. 13, 18, 20

and 21). The difficulty appears to be that the cortical plasm is incapable of holding the trichocyst threads after expulsion, for the threads may be easily seen as a cloud around the animal immediately after fixation, while the after-treatment always dislodges them in the cultivated forms, but not in the wild forms.

c. *The Macronucleus.* The structure of the normal macronucleus of *Paramæcium aurelia* was described by Hertwig in 1889 and the nucleus of *P. caudatum* agrees so closely with it, that further details are hardly necessary. It is an elongate, ellipsoidal body, usually with a smooth contour and without breaks of any kind save the minute impression made by the micronucleus. It frequently lies in a vacuole which is caused by the action of the fixing fluids, for in life the macronucleus is in immediate contact with the endoplasm. The contraction is probably in the endoplasm away from the nucleus rather than a contraction of the latter. Often there is a depression in the macronucleus due to the pressure of the contractile vacuole, and food vacuoles may also press against it, as Wallengren suggests, and distort it out of the normal proportions.

In its finer structure the macronucleus is granular with the irregular granules densely packed together, giving the appearance of a homogeneous mass.

d. *The Micronucleus.* The micronucleus is usually embedded in the material of the larger nucleus, but may be separated from the latter, even in the resting stages, by a considerable distance, while in the dividing stages it is usually separated. Its finer structure consists of a more or less homogeneous mass of chromatin frequently arranged in lines, while at one end is an accumulation of "achromatic" material in regard to which there is some difference of opinion. In size the micronucleus is about 11 microns, but in the different phases the size differs so that this characteristic has but little weight.

e. *The Contractile Vacuoles.* In the normal individual these are situated in the anterior and the posterior parts of the body, and about one-third of the length of the body from the ends. They are fed by radiating canals which are conspicuous in the living animals. The pulsation is regular as a rule, but this becomes

spasmodic after prolonged captivity under a cover glass, and the irregularity is an index of the ultimate disintegration.

## 2. *Structure of Paramæcium in Depression Periods.*

*a. Starvation and its Effects.* The periodic depressions which were noted in the experiments, and which appeared at more or less regular intervals (viz: about every six months) were noteworthy because not always accompanied by the same type of degeneration as that characteristic of starved forms.

A most comprehensive study of the structures of starved *Paramæcium* was made by Wallengren, while various observers have called attention to the characteristic vacuolization which the cell protoplasm undergoes during starvation or at degeneration periods in any culture. In general, Wallengren found that the animals first use up the food material that is stored in granular form, in the endoplasm, and that when this reserve is used, the animals in lieu of other food, burn up first their endoplasm and then the cortical plasm. There results from this destruction, great vacuoles in the cell body which increase in size until the entire organism is distorted through the pressure of one confluent, or two or three great vesicles. Wallengren obtained his material by transferring the *Paramæcia* to tap water again and again, and thus ridding the medium of the customary bacterial food in a very short time. My own experiments to this end consisted in leaving the ciliates in a culture glass such as I have used throughout my experiments, until all the bacteria had been eaten and the culture medium had cleared. Thus a hundred or more individuals would be left for a period of a month or six weeks in the culture chambers where evaporation was prevented, and here they were watched daily until they ultimately died of starvation. While Wallengren's experiments were undertaken for the purpose of determining the effect of starvation upon all of the protoplasmic structures of these forms, mine were done for the purpose of studying the effects of such treatment upon the nucleus and endoplasm, and general vitality. Wallengren found the following effects in the protoplasm of *Paramæcium* after starvation for a period of from 8 to 10 days, which he designates as the "first



period" in the inanition phenomena: "All gastric vacuoles and food balls disappear during the first period. After this the small endoplasmic granules are used. As a result of this the quantity of endoplasm becomes much reduced. Toward the end of this time the living substance of the endoplasm itself is used, in part at least, to supply fuel for the continuing metabolism. Owing to the disappearance of the inclusions of the endoplasm and to the use of endoplasmic substance itself, the body form becomes more or less distorted or changed. But even in those individuals in which this has taken place and in which the form is considerably changed, the ectoplasm with its trichocysts, the contractile vacuole and the cilia are not altered in any noticeable manner. This shows, therefore, that in the first period of inanition the first materials to be used are the reserve stuffs which are normally utilized for the ordinary fuel (life processes). Only when all of the reserve material is used and when the endoplasm itself is first attacked, and only when all food whatsoever is gone, will other parts of the protoplasmic structures be attacked. When this time comes the second period is inaugurated." (Loc. cit., p. 87.)

In the second period of inanition there are more fundamental changes, and the remainder of the protoplasmic structures are involved. Ultimately the nucleus is affected and when this goes the organisms are doomed. Wallengren's conclusions as to this period are as follows: "The endoplasm, which at the beginning of this last period was already considerably reduced, now appears strongly vacuolized. The shining vacuoles which are probably filled with the products of degeneration of the endoplasmic contents, may attain to a considerable size. Along with this vacuolization the ectoplasm becomes more and more absorbed and as a result of this, the trichocysts are drawn into the endoplasm streams and are probably digested. Along with them the small papilliform swellings on the outer surface disappear, and with these the small granules which in the living animal stain with neutral red. The contractile vacuoles and their feeding canals become reduced in the same proportion as the thinning of the ectoplasm. A number of cilia are probably absorbed as a result of the decreasing size of the whole animal, and the remainder of



them are shorter than the normal. Owing to the inner changes the whole organism may at this time be so modified that it is unrecognizable."

"Thus during the continued inanition of the body, first one part and then another becomes absorbed, first the endoplasm, next the ectoplasm, the trichocysts and the cilia in part, all to maintain as long as is possible the vital functions. In the meantime, however, the nucleus has not escaped without changes as follows:" (loc. cit., p. 98) . . . "In the inside of the macronucleus a rounded mulberry-like mass is developed. Its alveolar structure has changed at the same time, and in the center there are usually one or two small central bodies (Binnenkörper). The high pressure which is developed in the decreasing body form and due to the enlarging vacuoles, causes the nucleus to become greatly deformed and compressed. The various parts of the nucleus are broken up into fragments which may probably be used more or less as food (?). Of the former large macronucleus there is now left unchanged only the nuclear body which has been formed and this lies between the broken down nuclear parts." (Id., p. 112.) . . . "In the micronucleus no destructive changes are manifested during the hunger degeneration. It is the one part of the body which is apparently not affected by the conditions of the experiments, a not unnatural result considering the importance which this organ of these cells has in rebuilding the macronucleus after conjugation. Of all organoids the micronucleus would thus seem to be the most important of the cell." (Id., p. 114.)

These careful observations and clear results of Wallengren, most of which I have been able to verify, offer a good basis for the comparison of structures obtained in the different stages of the life history of *Paramæcium* (cf. Figs. 22 and 23). We may distinguish two types of degeneration changes in the series from the start to the finish. One set accompanies starvation, and was characteristic of the first two periods of depression, the other accompanies physiological depression of a different type at the last two periods. In the former the changes in structure had to do mainly with vacuolization of the endoplasm and rupture of the macronucleus, while in the latter the endoplasmic portions were

degenerated in a different way. The ectoplasmic parts and the micronuclear structures were not affected until the last depression period.

The first clearly marked period of depression came in July, about six months after the cultures were started. It was characterized by a well-defined reduction in size (down to 109 microns; see Fig. 3), and by vacuolization of the endoplasm while the ectoplasm did not appear to be much involved. Many of the individuals were characterized by great vacuoles similar to those in starved forms, which distorted the body almost out of recognition, in others the nuclei were fragmented into two or three parts, and in all there was a marked absence of the larger food granules and gastric vacuoles which characterize the normal animals, and this, notwithstanding the fact that bacterial food was present in abundance (see Studies I). As stated in these Studies (III) the organisms under these conditions still take food and in some cases the endoplasm appears opaque with the undigested food balls, but the decrease in size continues and the endoplasmic vacuolization is not prevented by the presence of the food. It is the digestive function, apparently, which becomes ineffective at such periods, and if this is a correct assumption, this function can be stimulated, as I have shown by the experiments.

Identical results were obtained in the period of depression in December, 1901, a depression which was again overcome by the use of beef extract, while the individuals of the series which had been continued on the hay diet, all died. These became smaller and smaller, and again gave morphological indications of starvation, notwithstanding the fact that the individuals which had been stimulated with the beef extract were living and reproducing normally in the same food medium. They became much reduced in size, the endoplasm became distorted with vacuoles, and they died with absolutely no indication of disease through parasites.

These observations show, therefore, that starvation effects may be produced even though the animals are living in a medium rich in food. It is trite to say that to prevent starvation we must have not only food but the ability to digest and assimilate it, yet common as this observation is, it is important in the present connec-

tion and involves a factor which cannot be overlooked in any discussion on old age.

In the June period, as stated previously, the same conditions were not observed, for the organisms, in part at least, had been treated with the beef extract every week during the first three months, since the previous period of depression. The division rate began to run down in the case of the B series in April, in the A series in May, and in all of the material that had been continued on the beef, the characteristic structure was a densely granular endoplasm (Fig. 7). In the specimens that had not been treated with the beef since the preceding December, this character of the endoplasm was not noted. These unstimulated individuals died out in about the 508th generation (B series) after becoming much emaciated and reduced in size, and with reduced nuclei. The nature of the protoplasmic changes is indicated, in one case at least, in Fig. 14. Here the macronucleus has entirely disappeared, not even a granular trace remaining, while the endoplasm is crowded with vacuoles of considerable size. The micronucleus is slightly hypertrophied and has a very peculiar outer membrane within which the chromatin and achromatic material lie in what appears to be the real nuclear membrane. The dense granules characteristic of the beef-fed individuals are absent. The unstimulated A series did not die out until about two weeks later. At the time when the B individual described above died (May 12) the unstimulated A series was characterized by somewhat reduced size, a declining division rate, and absence of the dense protoplasmic granules. In the stimulated A series, on the other hand, (A1 and A2) of about the 560th generation, the structures were normal, gastric vacuoles were numerous and divisions were frequent. Towards the end of June, however, when the A series nearly died out in the 620th generation, the conditions were very different. Fig. 7 is from a specimen in the 615th generation. Its size is below the normal; its endoplasm is choked up with granules and there is no trace of vacuoles save the contractile vacuole near one end. The macronucleus is definitely granular, and its contour is irregular as though devoid of nuclear membrane. The micronucleus is elongate and spindle-formed. The ectoplasm is



not deformed and save for the absence of trichocysts it appears to be normal. This was the condition of the protoplasm when the usual large number of culture individuals was reduced to 6 A's and no B's, and a condition from which the A series were rescued only with the greatest difficulty by the use of pancreas extract. Figs. 8, 9 and 10 represent individuals that had been in extract of pancreas for 48 hours, and then transferred to hay infusion. They are identical, therefore, with the individuals that lived and carried the race to the 742d generation. In these forms the endoplasm in most cases is normally vesicular in the center and gastric vacuoles are common, while the ends alone still retain the dense granular aspect.

From this time until the race died out the division rate was sluggish. The conditions of the protoplasm in the later individuals was decidedly characteristic (Figs. 17, 19, 20, 21 and 22). Throughout the fall, individuals would appear with densely granular protoplasm, which is invariably the sign of death, unless the animals are stimulated in some way. In such forms the macronucleus may or may not be normal, whereas the micronucleus as a rule becomes hypertrophied and the ectoplasm full of great vacuoles. Fig. 22 is a good representation of the conditions at this time. The endoplasm is apparently normal; there are food vacuoles and endoplasmic granules, and vesicular structure, but the micronucleus is spherical and vesicular, has lost its usual place in a niche in the macronucleus and shows evidence of granular modification of the previously homogeneous chromatin.

The sister-cell of the one pictured in Fig. 22, and one of the two oldest of the A series (742 generations), showed the following points while alive: "A12 was alive this morning and was picked out for examination. It had two contractile vacuoles situated dorsally and close together. The astral canals were absent; in their place was a row of dorsal feeding canals, such as those characteristic of the more generalized holotrichida (*e.g.*, *Cblamydodontidæ*). The rest of the body contained eight or ten large vacuoles not contractile. The macronucleus was slightly hypertrophied, and visible, indicating the approach of disintegration.



The papillæ of the cuticle were plainly visible and what I have taken to be apertures of the trichocysts were more or less numerous. (This is shown in the preserved sister-cell, Fig. 22.) A few trichocysts remained in the cortical plasm, but there were many vacuoles in this layer indicating that when the trichocysts were discharged they were not re-formed. The peristome was normal and the mouth had a vigorous oral membrane. The size was large, fully as great as any of the preparations that had been made at any time during the 742 generations. Movements vigorous to slow, with a tendency on the part of the animal to remain stationary."<sup>1</sup>

It was while the organisms were in this structural condition that the many attempts to rejuvenate the race were made as described in the previous pages, and it was in this condition of the protoplasm that the race finally died out from exhaustion. Before dying, however, the individuals, as indicated in the above paragraph from my notes, were of full size and were filled with gastric vacuoles and partly digested food, while the body form was normal, (compare Figs. 2 and 21).

*It must be admitted that these forms were capable of individual growth at this period and, since the macronucleus was normal in the last individuals while the micronucleus was considerably changed, it must be further admitted that the vegetative metabolic processes were presumably reinvigorated; on the other hand, the functions of reproduction; that is, of division, were degenerated possibly, if not probably, because of the apparent degeneration of the micronucleus and of the cortical plasm, whose functions were not reinvigorated by the artificial means which were tried.*

#### IV. GENERAL DISCUSSION.

Although only a beginning has been made to determine the objects for which this series of experiments was started, it is advisable to bring together the results thus far attained and to see how they conform with the *a priori* conceptions which were current at the outset of the experiments.

<sup>1</sup> From my note book.

It is not out of place to consider first the initial objects of the undertaking, although at the risk of again repeating what has been often stated.

1. The first aim of the experiments was to get light upon the general phenomenon of conjugation and through this, upon fertilization in general.

2. To determine whether conjugation is imperatively necessary for rejuvenescence.

3. To determine whether artificial rejuvenescence is possible.

4. To determine the conditions, antecedent and subsequent to conjugation.

5. To determine, if possible, the significance of rejuvenescence.

6. To determine, finally, whether protoplasm in these simple forms is capable of indefinitely continued life without conjugation, or whether it is subject to the conditions of "old age."

On none of these points can a definitely positive answer be given, and further experiments must be undertaken to clear them up. The fact that, after a continuous cultivation of 742 generations, covering a period of 23 months, the race died out apparently from exhaustion, shows that *under the conditions*, continued life was impossible, and if this conclusion, which seems to be the only one justified by the results, be granted to obtain in nature, then we must agree with Maupas and others that the indefinite continuance of life without conjugation, is improbable.

### 1. *The Conditions of the Experiments.*

The question has been raised whether the conditions under which the experiments were undertaken were in any way abnormal to *Paramæcium*, and whether, from the results obtained, we are justified in carrying the conclusions to the free-living forms, and to similar types in general.

It might be objected that the space allowed was inadequate; or, that the light conditions were abnormal; or, that the water would get foul; or, that they were given only one kind of food; or, that they were subjected to pressure. If we examine these objections critically we shall find that they have little basis.

Let us consider first the matter of space, for this involves some of the other objections, viz: pressure, volume conditions, and the

like. The actual amount of water that was used for each isolated individual was one-half a cubic centimeter. This was contained in a small chamber consisting of a hollow-ground slide, two glass supports about 3 mm. thick, and a thin glass cover. The *Paramæcium* had ample room, therefore, for free movement, and an actual depth of water of more than an eighth of an inch. Pressure, therefore, was out of the question. In such a slide chamber individuals were kept (*i. e.*, extra individuals from the "stock") for periods considerably longer than six weeks without change of water, showing that the mere quantity was sufficient in order to keep the animals alive. Foulness of the water, accumulation of carbon dioxide, lack of oxygen, etc., were all guarded against by the almost daily transfer of the culture individuals into fresh hay infusion. The salt content of the water remained practically constant, for fresh hay infusion was used each time with the same amount of hay from the same source while the weekly analysis of Croton water shows only minor fluctuations in the small quantity of salts in solution. The gradual decrease in vitality cannot be attributed to these causes, a similar phenomenon being a matter of common observation and noticeable in any culture of protozoa, no matter how large the vessel, nor what the species. The light conditions were similar to those in any laboratory, the culture vessels being kept before a window with north exposure.

In regard to the possible objection that the *Paramæcium* obtained only one kind of food, and therefore that the conditions were abnormal in this respect, it may be stated that such a condition of treatment is a *sine qua non* of the experiments, and the only possible means of controlling the results, and as I have demonstrated, it is by a change of diet, including salt constituents, that the periods of depression are overcome. This objection, therefore, begs the question of an object of the investigation.

It seems quite unnecessary to repeat again that the only normal life possible to *Paramæcium caudatum* is in the ponds where it is subject to the changes in chemical composition of the water, to the exigencies of drought, of heat, of freezing, and of rest by encystment or lack of food. In the laboratory the protoplasmic activities get no rest, but day after day they are maintained at the



optimum rate and such conditions can by no stretch of the imagination be called identical with those of the ponds. Yet the "normal conditions" may, after all, be but a matter of definition. If we leave a hay infusion to stand exposed to the air, *Paramæcium* will ultimately appear in it, and will ultimately die out from it. The appearance and disappearance cannot be called artificial, it is as much normal for *Paramæcium* to appear in such an infusion as it is normal for the bacteria upon which the animals feed to be there. City life for man may be called an artificial life as opposed to the "normal" original or pastoral life, but it is no less normal now than the primitive life was, even if it is found that the average length of life is shorter for urban than for country-dwelling people. The course of human life, or the history of the race, physiologically speaking, is no less normal for being rapid. In the same way we may argue for the race of *Paramæcium* and its life in the culture chambers of the laboratory. The life pursues a normal course, although possibly faster than in nature, and the ultimate results obtained in cultures may be confidently expected to obtain sooner or later in the natural habitats. Seven hundred and forty-two generations represent a long time for organisms to live and develop in a medium that is not normal, and the mere fact that they do so live is sufficient evidence to prove the point. It seems to me, therefore, perfectly legitimate to take the phenomena of vitality in *Paramæcium* in culture as practically identical in outcome with the phenomena in natural surroundings, and as indicative of what goes on in living protoplasm under "normal conditions."

Looked at from this point of view, the experiments teach (1) that a given form together with the race derived from it will exhibit periodic depressions in vital activity; (2) That such depressions can be overcome by artificial means (and probably but not surely, in nature by opportune changes in the immediate environment). Further than this, however, the experiments teach, (3) that these depressions are not all of the same type, nor due to the same causes. They give reason for the belief that periods of depression may ensue wherein different functions give out, and that when this occurs, as for example when the cortical



plasm and micronucleus show evidences of degeneration, all of the experiments that we may try to artificially reinvigorate them, will probably be futile. This may indicate one of two things, viz: that under natural conditions changes in immediate environment would be insufficient to rejuvenate when the organisms are in this ultimate state of exhaustion, unless, indeed, the experiments failed to eliminate all of the chances such an organism has in nature; or, conjugation is a necessary condition of continued protoplasmic activity.

I am inclined to the belief that some material might ultimately have been found which would have helped the *Paramæcia* over this period of extremity and would have stimulated micronucleus and cortical plasm to continued work. The failure to find it, however, indicates a like difficulty in nature and makes the *a priori* reason most probable that the phenomena with which we are familiar, namely, the processes of conjugation, have been essential in maintaining the races of *Paramæcium* up to the present time, and in keeping them from extinction.

## 2. Does Protoplasm Grow Old?

The above considerations lead to the discussion of age in a simple cell organism. In higher forms old age is manifested by the gradual weakening of the vital functions, waste matters are inadequately disposed of, or are retained in one form or another in the cells and tissues; this involves the physical impairment of organs and enhances the difficulties of their functional activities until, by the accumulation of such mutually aggravating processes, the organism ultimately dies of "old age." In *Paramæcium* there is little morphological evidence of the onset of old age, although, if we accept the impairment of the vital functions as an index, we must conclude that diminution of the division rate, decrease in size, etc., are evidences of this phenomenon in protozoa. So far as the accumulation of waste matters is concerned, there is morphological evidence to indicate that this takes place more frequently at periods of depression. There was no sign of the crystals which frequently accumulate in the protoplasm of various protozoa, and in the last specimens of the race (742d generation) both endoplasm and macronucleus were normal in structure.

The surest evidence of what may be considered old age in this form, was therefore, functional, and was expressed by diminished division rate and by the increased frequency of abnormal binary fission. Abnormal division, as a matter of fact, like nuclear hypertrophy, may occur at all periods and marks some particular weakness of the single individual; occurring more frequently, however, at certain periods of depression, such abnormalities give evidence of general protoplasmic weakness. The various types of incomplete division are very instructive and a more prolonged study than I was able to give to them might afford positive evidence of the nature of the pathological changes involved. In some of the specimens which I obtained during periods of depression, the macronuclei and micronuclei appear normal; in others there is a macronucleus in each of the daughter individuals, but the micronucleus is undivided; in others the macronucleus is divided but remains in one individual, the micronucleus is undivided and remains with the original macronucleus, while the daughter individual has no trace of nuclei. In all cases, finally, of pathological division the cortical plasm appears abnormal and vacuolar, while the endoplasm is very frequently disintegrated and abnormal (Figs. 25 and 26).

While these observations are too few to permit far-reaching conclusions, they are sufficient to indicate that some protoplasmic changes have taken place, and further, that the cortical plasm has become modified in some way. Indeed, the inability completely to divide may be accounted for by the loss of vitality in this particular part of the protoplasm, for in the majority of cases the initial stages of division are safely passed, the final separation alone being retarded and usually omitted altogether, so that monsters of three or four individuals may be formed through the continued incomplete division of the original degenerate specimen (Fig. 25). As is well known, the cortical plasm is the seat of the myoneme formation, of the cilia, and of other motile organs, and, in general, may be said to possess kinetic or motor functions. That this portion of the protoplasm is subject to change is shown by the fact that at certain times the outer protoplasm becomes

sticky or plastic and to such an extent that two individuals upon meeting, fuse together in plastogamy. This, which I have termed elsewhere the "miscible state," may be so marked that groups consisting often of from five to eight aggregated individuals are occasionally seen. It is analogous, apparently, to the plastogamy so often seen in the fresh water testacea such as *Diffugia* or *Arcella*, which Schaudinn has recently shown to have no connection with conjugation in these instances. In *Paramœcium* during this miscible state, conjugations are for the only times possible, and many complete conjugations are found together with the fused multiple individuals. *There is no doubt, then, that the cortical plasm changes in physical condition, and there is equal reason to believe that at periods of depression when these abnormal divisions are more frequent, the cortical plasm shows degenerate conditions, or, possibly, a condition of old age.*

There is therefore some significance in the fact that the cortical plasm gives out; some significance connected with the diminishing division rate and with advancing old age as evidenced by diminishing activity.

While there may be some uncertainty as to whether the decreasing vitality of a race of *Paramœcium* is evidence of normally decreasing functions indicative of protoplasmic old age, or of some other cause of degeneration, there is absolutely no reason to believe that it is due to a parasite of any kind, nor to any harmful substances in the medium in which they live. In the earlier periods of depression there seems to have been a gradual loss of powers connected with metabolism, and of something which was vitally important to the race, for unless the individuals were stimulated, they inevitably died. This was strikingly demonstrated in the period of depression in December, 1901, when a number of individuals of the regular series were continued on the usual hay infusion, while others were treated with beef extract for 24 hours, and still others with salts of different kinds for not more than 30 minutes. The non-stimulated forms showed increasing sluggishness and depression, and all died in the course of two weeks, while the sister-cells which had been stimulated, lived with varying fortunes until a year from then (see Diagram I). The pertinent



questions may be asked was it old age from which the organism died? and, if so, what form did it take? They were fed daily with the same food upon which the stimulated sister cells thrived, but they could not assimilate it and would not grow nor divide. In similar cultures which had been carried to a like point by previous observers, the entire race died, and although no evidence of structural degeneration was evident, it has been taken for granted that their organisms died from exhausted vitality, or in other words, of old age. In my cultures there was some evidence of degeneration, especially in the endoplasmic structures and in the macronucleus.

The fact that stimulation was successful in carrying the race through this earlier period of depression indicates either that the conditions are not the same as those accompanying old age in metazoa, or else that such conditions may be satisfactorily overcome. I believe the conditions are more or less the same in both cases, and that in senile *Paramæcia* certain functions have become retarded, possibly by the accumulation of useless protoplasmic elements too minute to be detected, or by some less mechanical cause connected with the molecular structure of protoplasm and which, therefore, affords no morphological evidence of change. Such an hypothesis would explain the difference in length of time required to get positive results in the stimulation experiments. For example, in August, 1901, after the race had been on hay infusion continuously for 7 months, it was necessary to keep the single individuals on beef extract for three weeks before they would live again in the hay infusion. But in December it was necessary to keep them on the stimulant only a day or two to get the desired result. The short treatment at this period sufficed, because they were not allowed to become weakened to the same extent as in the preceding period of depression. This result points to some physical condition of the protoplasm, possibly to the accumulation of some protoplasmic product or products which lead to diminished vigor and to death. Reinvigoration after a more or less prolonged treatment with the beef extract and stimulation by this and other means indicates that such materials are disposed of, or, more generally speaking, and to use a phrase which



Wilson in *The Cell* attributes to O. Hertwig, that the condition of stability is changed into one of protoplasmic lability.

While such an hypothesis accounts for the first two periods of depression, it fails to account for that of June and of December, 1902. In the interval between June and the preceding December, the race in part, had been treated weekly with beef extract until the first of April, after which the organisms had been fed with the usual hay infusion. In June they began to degenerate, and from this time on, treatment with the beef extract was futile, and the race was finally saved only by using extract of pancreas and of brain. This, however, gave only temporary relief and complete activity was never again recovered and the division rate remained below the average, until the race finally became extinct in December, 1902, and this despite the fact that, morphologically, the endoplasm and macronucleus were restored.

Was the last period of depression running from June until December, 1902, an expression of old age? From the structures of the organism and their behavior, there is no doubt that the ailment at this period was different from that of the earlier periods of depression, and there is no doubt again that the remedies which had succeeded at the earlier periods failed completely at this. The final depression of vital activities may be accounted for by one of two assumptions: (1) There was an accumulation of waste material of a different kind from that of the earlier periods, or a different physical condition, and a weakening of different functions, or (2) certain elements in the protoplasm endowed with a given potential of activity used up that potential and failed to recover it by artificial stimulation. Or a third hypothesis may be conceived which embodies both of these. The morphological structure at the final period shows that some different elements of the body were involved in the last period of depression, and that the elements which had given out in the previous periods were satisfactorily reinvigorated even in the last individuals of the race. Thus the micronucleus and the cortical plasm showed unmistakable signs of degeneration in the last few individuals of the race, while the endoplasm and macronucleus were perfectly normal in appearance, and metabolism, which these elements of

the cell appear to control, seemed to be equally normal, since the organisms were of full size, while the endoplasm was full of partly digested food. It appears, then, that the experiments were successful in reinvigorating the elements of the cell that had given out in previous periods of depression, but that other elements were now involved which all my experiments failed to reach. Here a more deeply-lying malady had to be met, and the experiments being unsuccessful in meeting it, the entire race died out. *This series of facts appears to warrant the assumption that there is a fundamental difference in the protoplasmic elements which go to make up the body of a protozoon, one of which is to be compared with the somatic cells of metazoa, the other with germ cells; the one connected with the vegetative functions of metabolism, the other with reproduction; the one may give out and so lead to "physiological death" (Hertwig) or it may be restimulated; the other may give out and so lead to "germinal death" of the race.*

It is not outside the range of possibility that the last depression period might have been overcome by some suitable experiments, and the fact that we did not succeed in finding a suitable stimulant does not justify us in assuming that this period represents the last vital spark of this protoplasm, any more than we are justified in assuming that the earlier periods of depression represented this condition. If, however, some element or elements of the protoplasm become exhausted and all experiments to replace them fail, then we might justly speak of exhaustion or "old age" of these elements of the protoplasm and affirm that old age in one form, characterized the organisms during the first two periods of depression, while it took another form in the final period.

### 3. Conjugation and Rejuvenescence.

"Old age," then, appears to be a natural condition of living protoplasm and we may ask, is there any experimental evidence to show that this condition may be overcome by natural means?

It has been generally assumed by biologists that conjugation brings about rejuvenescence in the conjugating individuals, and so imparts to the ex-conjugants and to their immediate descendants a high potential of vigor. During the process of conjugation

there is a complete change of materials or of protoplasmic make-up, and a thorough "reorganization," to use the excellent term proposed by Engelmann. Not only is there a new conjoint micronucleus with its chemical compounds derived from the union of two nuclei from individuals of diverse environment, but the endoplasm and cortical plasm must receive new materials through the disintegration and the absorption of the old macronucleus, and of at least three-quarters of the old micronucleus. If, as I have long maintained, there is a specific "kinetic" substance in the protozoon nucleus, a substance which in the centro-nuclei forms the division-center and which is found in the micronucleus of *Paramæcium*, then the cytoplasm of *Paramæcium* must receive a certain amount of "kinoplasm" at each period of conjugation and from the experiments, enough to carry the race through a complete cycle. In my cultures such reorganization by conjugation was prevented in the straight line of the experiments, and the only opportunity for reorganization came with the change in diet. This, indeed, seemed to be operative for some time, but ultimately failed, as we have seen. In the stock material, however, material left over after the individuals had been selected for the cultures, conjugation experiments were frequently tried during the course of the experiments, and the results have been given (Studies I). Some of the results are very suggestive in the present connection, for it was found that only a few of the ex-conjugants continued to live, approximately 6 per cent of them. This result may be due, as I have previously stated, to the fact that both of the gametes had been under identical conditions of food, etc., and no new substances were formed by the union of similar nuclei and protoplasm. Or the result may be due to the fact which Stevens<sup>1</sup> calls attention to, that conjugation is an exhausting process, and that, being weakened through long cultivation in cultures, these ex-conjugants did not have sufficient vitality to recover. This suggestion does not set aside all of the difficulties, however, for we have still to explain the large number of cases where the ex-conjugants have lived

<sup>1</sup>N. M. Stevens. Further Studies on the Ciliate Infusoria, *Lichnophora* and *Boveria*. Arch. f. Protistenk., III, 1903.



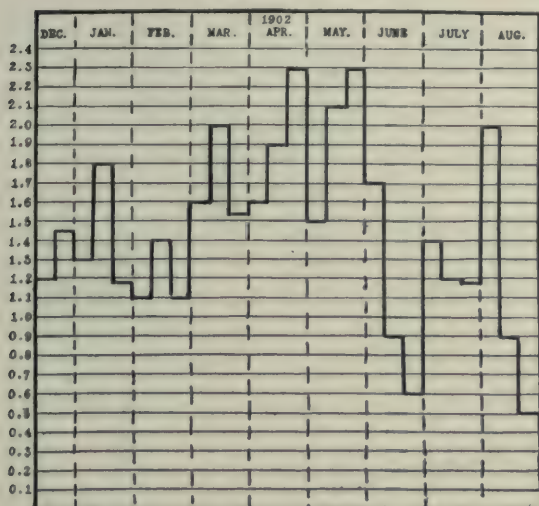


DIAGRAM II.

Complete history of the endogamous ex-conjugant by ten-day periods. Ordinates and periods the same as in Diagram I

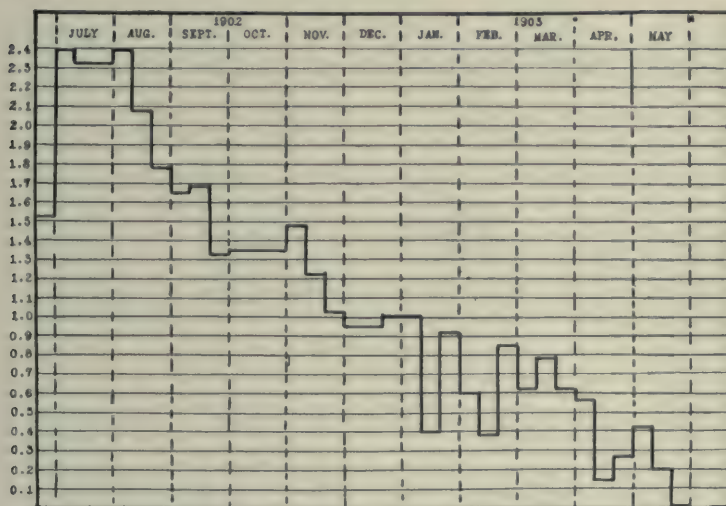


DIAGRAM III.

Complete history of the third series (C) by ten-day periods. Ordinates and periods the same as in Diagram I. As in Diagram I, this curve represents the average division rates of four lines of individuals.



and reproduced for from 8 to 20 generations, and with apparently well-organized bodies.<sup>1</sup>

One of these successful cases was an ex-conjugant from an endogamous union of two individuals which were separated by not more than eight or ten generations from the ancestral A in the 354th generation of my cultures. The other ex-conjugant died out in the 11th generation, while the successful one ran through 376 generations before showing signs of debility. It went through eight months in culture without being stimulated, and died out finally at the end of 376 generations, which was exactly three generations less than the life of the third series of *Paramæcium* (C series) which I started on June 18, 1902, and carried along in culture until May 30, 1903, when it died out in the 379th generation (see Diagram III).<sup>2</sup>

Unfortunately, this ex-conjugant has not an absolutely clear record, for the first day after the pair had separated, I placed them both in beef extract for 24 hours (December 9, 1901). This experiment had failed a number of times, and I had no reason to believe that it would succeed this time, and, as stated above, one of the two ex-conjugants thus treated died after eleven generations. Although at first I attributed the successful result to this treatment, I do not now believe that the beef extract had anything to do with the vigor of the race that followed, and believe that rejuvenescence was accomplished by the conjugation and nothing else. This conclusion is based upon the following facts: (1) Other ex-conjugants similarly treated with the beef extract failed to live; (2) *the non-conjugating individuals of the regular series which were treated with the beef extract at the same time*

<sup>1</sup>See Studies I, table of conjugations opposite p. 174.

<sup>2</sup>We have, then, the interesting coincidence of an individual running through 354 generations in culture, conjugating with one of its own close relations, and then, as an endogamous ex-conjugant, running through 376 generations more, a total of 730 generations. Against this we must set the 742 generations of the main culture series, and the 379 generations of the third series (C). The close connection between the 379 and 376 is very significant, and were it not for the fact that the first two series, A and B, were at a fatal period of depression at the end of 200 and 190 generations, we might conclude that 370 more or less is the normal length of life of *Paramæcium* in culture.

had a lower division rate and died out before May 5; that is, after running five months (cf. Diagrams I and II). It follows, therefore, that something was operative in the ex-conjugant that was absent in the stimulated form, and this something could be nothing else than the reorganization which follows conjugation. The accompanying curves show that the periods of depression and death which menaced the regular series in December, 1901, and again in June, 1902, were not paralleled in the cultures of the descendants of the ex-conjugant, and the conclusion is obvious that conjugation provided some stimulus which enabled this line of *Paramæcium* to live through periods in which the allied races were saved only by vigorous treatment and stimulation. There is no doubt at all that, had I tried to revive the race of the ex-conjugant by beef extract at the end of August, 1902, I could have done so, for there was nothing serious in the nature of the depression at this time, when I allowed them to die without making an effort to save the race. It is now a matter of deep regret to me that I did not try to save them, and see if they would live beyond the time when the allied lines died out in December, 1902. Had they done so, it would have been still more convincing proof that conjugation does actually rejuvenate and overcome the conditions of so-called "old age." I believe that the evidence which I have outlined above is quite sufficient, however, to establish this point, the one questionable factor being the beef extract, and even this, as I have shown, could have only a limited bearing and does not at all outweigh the positive evidence in favor of the conclusion.

Columbia University,  
April, 1904.

## EXPLANATION OF PLATES.

All of the photographs were taken by Dr. Edward Leaming from permanent preparations of *Paramecium caudatum*, stained with picro-carmine. All are equally magnified and the relative sizes represent absolute differences.

## PLATE I.

Figs. 1 and 2. Two normal specimens B series (107th generation and after three months of culture in hay infusion. These do not differ from typical *Paramecium* from the ponds, and have many endoplasmic vacuoles, alveolar protoplasm, and homogeneous nuclei.

Fig. 3. A typical individual of the B series during the first period of depression. The ectoplasm is fully as clearly defined, and as thick as in the largest forms, indicating that this portion at least, has not suffered from degeneration, a result differing from that in starved forms. (Compare Figs. 23 and 24).

Fig. 4. An individual from the B series in the 306th generation, stimulated with beef extract in August, fed continuously with hay infusion for three months until killed. The endoplasm is filled with gastric vacuoles and with partly digested food, the dissociated or "labile" condition of the endoplasm shown here is characteristic of *Paramecium* under normal conditions.

Fig. 5. An individual from the A series during the third cycle (550th generation), and twenty-four hours after treatment with beef extract. The endoplasm is filled with gastric vacuoles, the macronucleus is normal, but the micronucleus has divided three times and a clump of six nuclei may be seen at the lower end. There is a tendency toward a denser structure of the endoplasm, especially at the two extremities, this being indicative of approaching physiological depression.

Fig. 6. An individual from the A series in the 560th generation. Treated 48 hours before fixation with beef extract. Gastric vacuoles are abundant in the upper portion, but in the lower part the characteristic density which marks the climax of physiological depression is shown, *i. e.*, an apparently general "loading" of the protoplasm with inert material.

Fig. 7. An individual from the A series in the 615th generation killed at a time of general depression. It shows the typical condensed appearance when the power of division is lost and leads to death after several days without division.

Fig. 8. An individual from the A series in the 623d generation (June, 1902,) and 24 hours after successful stimulation with extract of pancreas. The condition shown in Fig. 7 has been successfully overcome, and activity renewed by this treatment. This and the two following figures show stages in the breaking up of this dense, endoplasmic mass. The macronucleus is divided while the ends alone of the animal still retain the densely granular character.

Figs. 9 and 10. Two individuals from the A series 48 hours after successful stimulation with pancreas extract. The endoplasm is now in a "labile" condition, although the extremities are still dense. The individual shown in Fig. 10 is further advanced in recovery than that shown in Fig. 9, but both are sister cells of individuals that carried the race to the 742d generation.

## PLATE II.

Fig. 11. Two individuals of the A series in the 604th generation, two weeks prior to the fatal depression of June, 1902. These were treated with a weak solution of dibasic potassic phosphate (see paper) for 30 minutes and then transferred to hay infusion and killed 24 hours afterwards.

Fig. 12. Two individuals of the A series treated at the same time as the preceding, with a weak solution of magnesium chloride for 25 minutes. The protoplasmic structures are normal and the endoplasm has the typical alveolar appearance.



Fig. 13. Another individual treated with potassium phosphate. The micronucleus is separated from the macronucleus. At the lower end the focus is sufficiently sharp to show the characteristic papilli-form structure of the trichocyst spaces in the ectoplasm.

Fig. 14. One of the last individuals of the B series (504th generation) in which the macronucleus is entirely gone. The micronucleus is distinct, and has its chromatin massed near one pole. The place which held the macronucleus is marked by a large vacuole. There are no observations to indicate the fate of this macronucleus, the break at the left side indicates that it may have dropped out at some period, although this did not happen during the course of the treatment, because the same condition was observed during its life, and immediately after killing.

Fig. 15. An individual from the B series in the 502d generation after treatment with beef extract. The characteristic dense endoplasm is still present but there are many gastric vacuoles, while the micronucleus has divided three or more times and the daughter nuclei have accumulated at one end.

Fig. 16. An individual of the A series in the 602d generation treated for 25 minutes with phosphoric acid. It was transferred to hay infusion and killed 24 hours afterwards. The macronucleus is broken into fragments; the micronucleus has divided and one part (left center) seems to be forming a new macronucleus. (This individual offers the only evidence obtained of nuclear fragmentation and reconstruction through artificial means.)

Fig. 17. An individual from the A series in the 718th generation, killed October 20 after living six days without division. The endoplasm shows a general absence of the larger granules, indicating starvation; the micronucleus (dimly visible at the lower end of the macronucleus) is hyaline and without chromatin, evidently degenerated.

Fig. 18. Two individuals of the A series in the 602d generation, treated with a dilute solution of sodium chloride (see description), for 25 minutes. Transferred to hay infusion 24 hours afterward, and killed.

#### PLATE III.

Figs. 19, 20, 21 and 22. Four individuals at the end period of the A series. Note that in all of these the macronucleus is not abnormally large as compared with normal individuals. This was true throughout the entire race at this period and contradicts Hertwig's recent theory of the size-relations at periods of depression. Fig. 19 represents an individual in the 720th generation, unusually small and unlike the remainder of the culture at this time. Fig. 20 represents an individual in the 725th generation, with conspicuously dense endoplasm and macronucleus. The latter bulges out towards the observer and the effect of the ectoplasm about it is that of a special nuclear capsule. Fig. 21, an individual in the 741st generation showing the looser texture of the endoplasm, gastric vacuoles and other characters, indicating that these organs had been restored by stimulation. The micronucleus is hypertrophied, the macronucleus is normal. Fig. 22 represents an individual in the 742d generation, the oldest of the race. It shows the reorganized endoplasm, gastric vacuoles, and the like, but ectoplasm and micronucleus are degenerated. The former by vacuolization (note punctate appearance on right of macronucleus) the latter by hypertrophy and loss of chromatin.

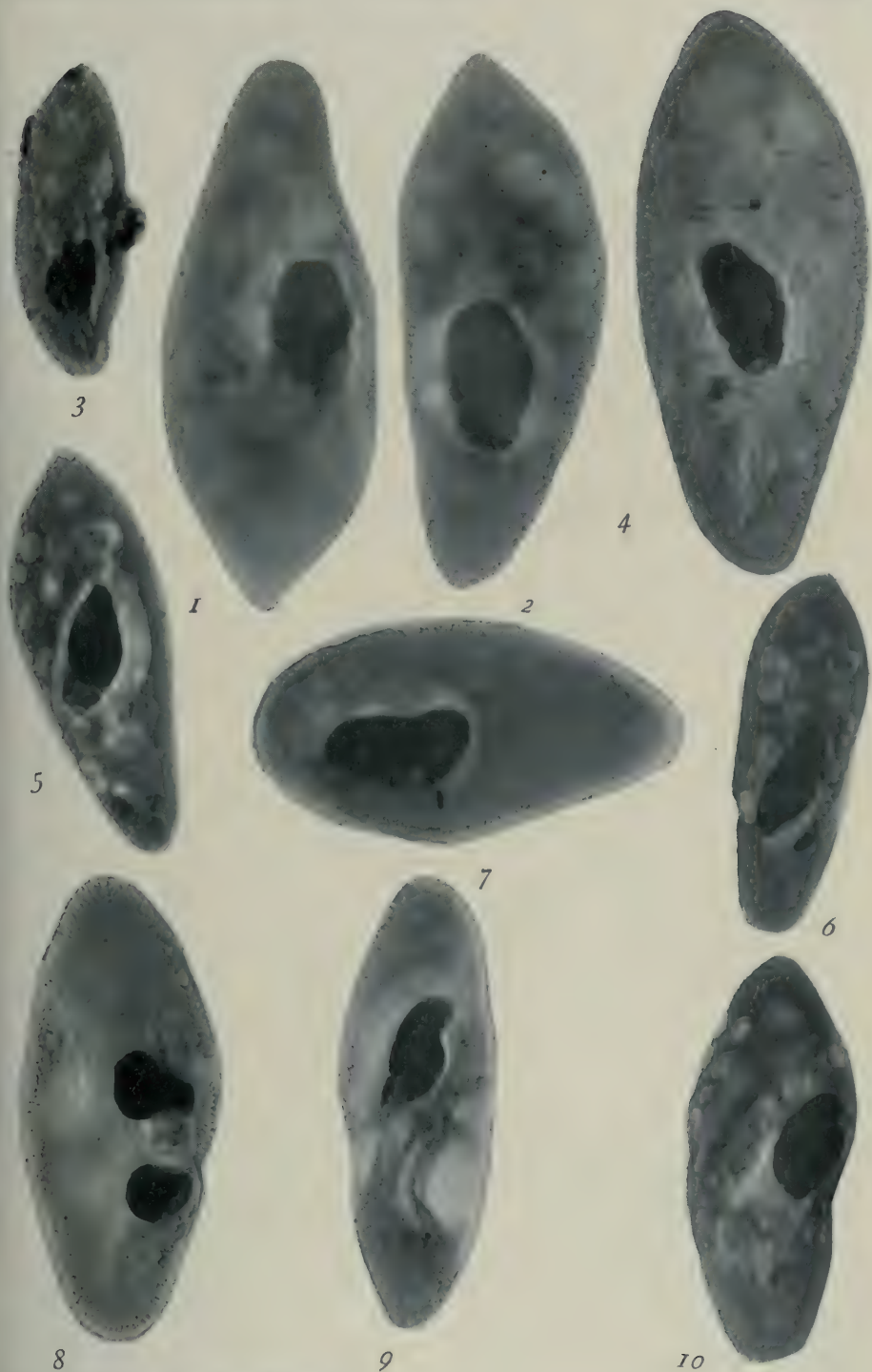
Figs. 23 and 24. These represent individuals which were starved for two and four weeks respectively. Those in Fig. 23 were fed on beef extract August 19, transferred to hay August 20th and left unchanged until September 19, when they were killed. The individual shown in Fig. 24 was not given beef extract, but was left in hay infusion for two weeks unchanged, when it was killed. In Fig. 23 the spots at the lower ends represent the micronuclei, in Fig. 24 the upper elongated granule is the micronucleus.

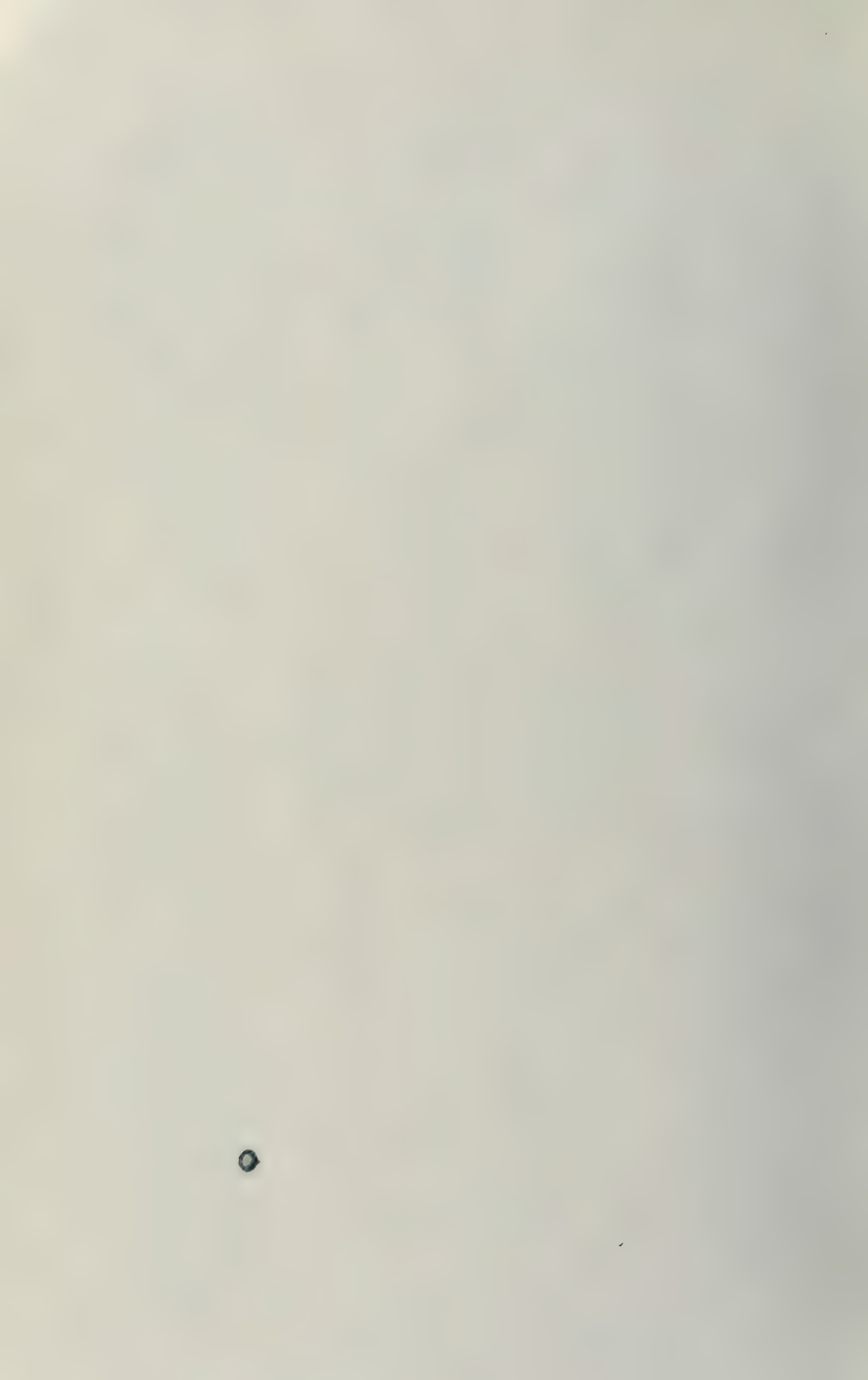
Fig. 25. A triple monster from an individual 72 hours after conjugation, with many nuclear fragments and evidence of two incomplete divisions.

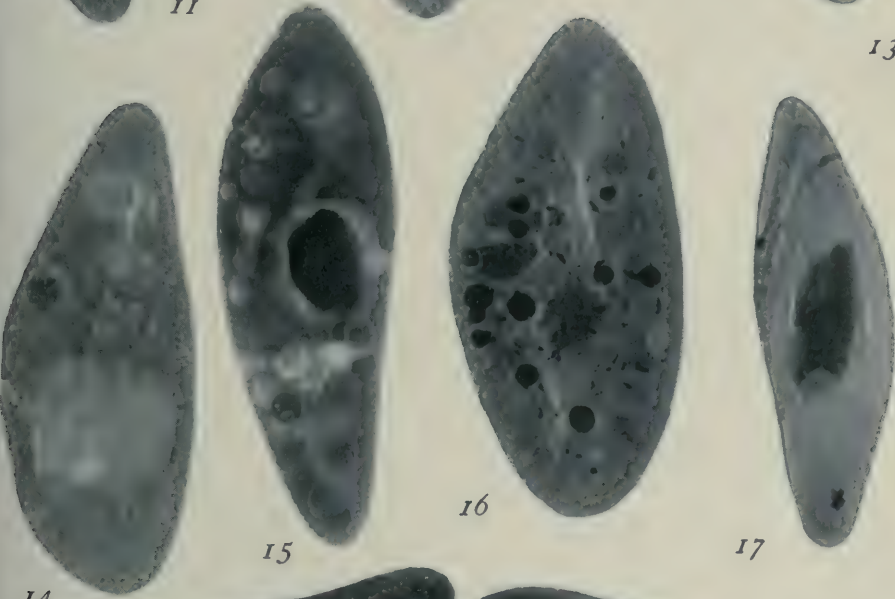
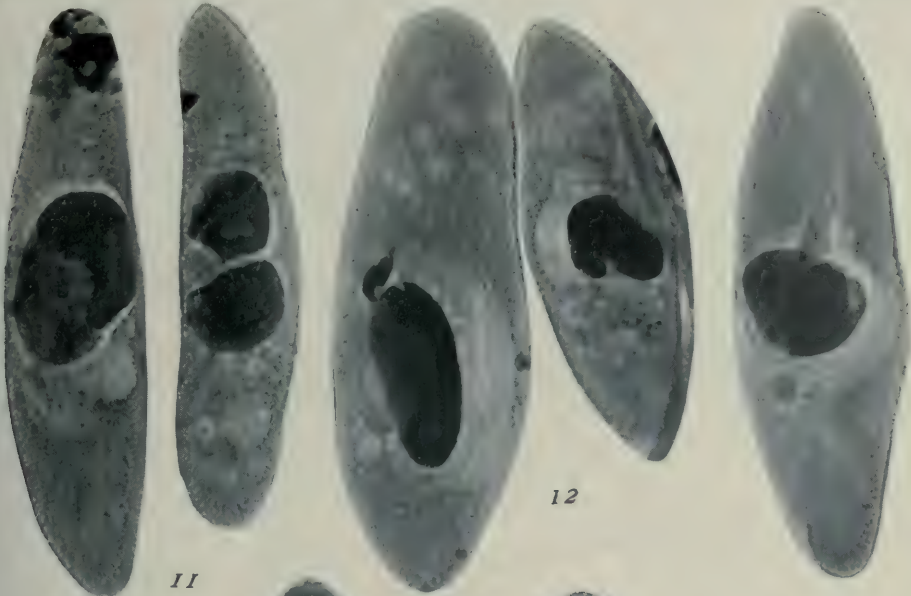
Fig. 26. A double monster from the A series in September, 1901. The micronucleus is undivided, the macronucleus is deeply cleft and the individual on the right has no trace of nuclei.





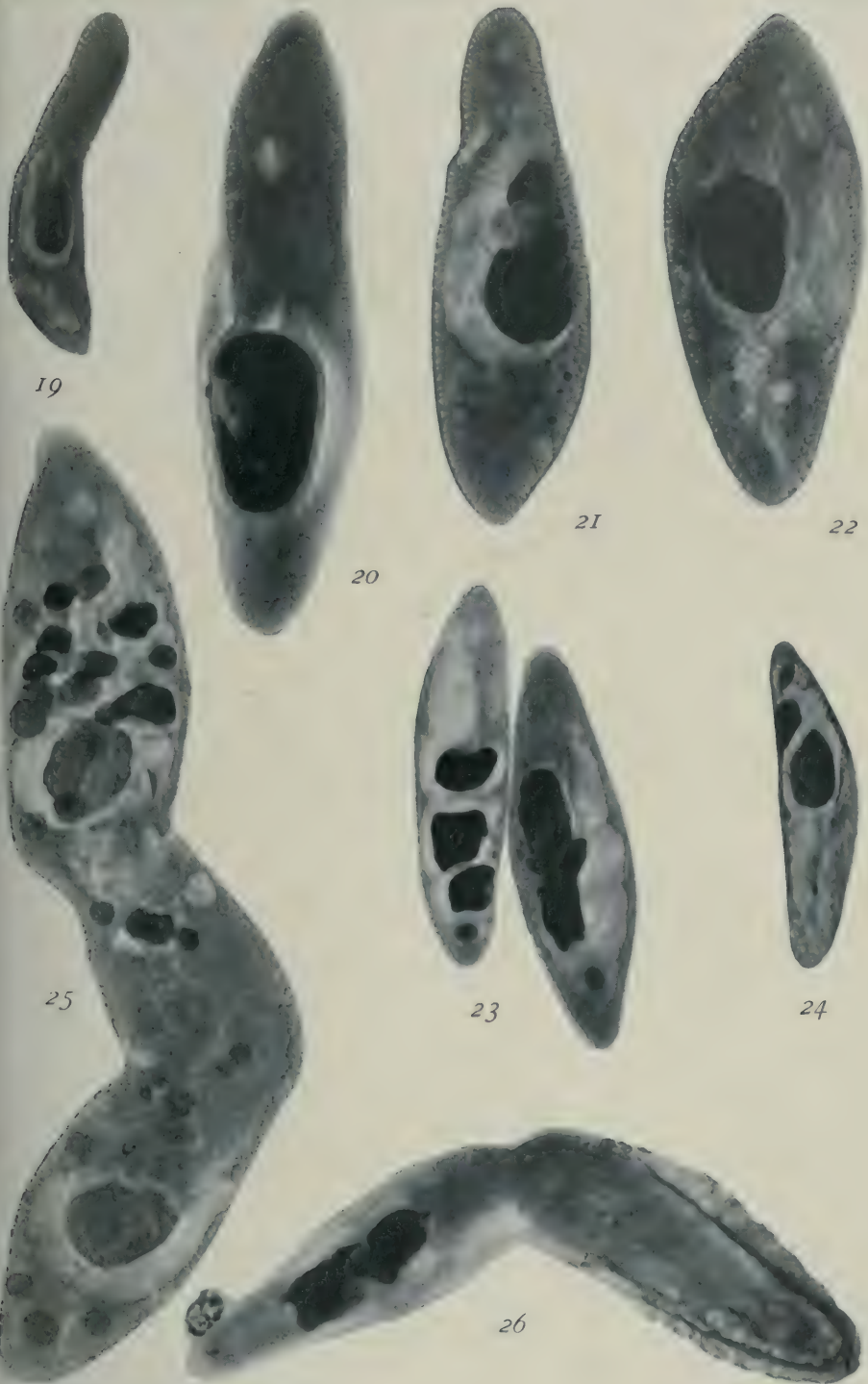














## STUDIES ON REGULATION.

### V. THE RELATION BETWEEN THE CENTRAL NERVOUS SYSTEM AND REGENERATION IN LEPTOPLANA: POSTERIOR REGENERATION.

BY

C. M. CHILD.

WITH 47 TEXT FIGURES.

#### A. INTRODUCTION.

It is beyond the purpose of the present paper to review the whole question of the relation between the nervous system and morphogenesis or the sustaining effect of "trophic" stimuli upon form. It is hoped, however, that the observations and experiments to be described, together with the interpretation offered, may serve to throw some light upon this most interesting, but difficult problem.

The question of the relation of the central nervous system to regeneration in the lower animals has been touched upon by various authors. As regards *Planaria*, a species which has been the object of study by many investigators, opinions differ to some extent at the present time. It is well known that in this form removal of the cephalic ganglia does not interfere with complete regeneration, the ganglia themselves being regenerated from portions of the nervous system which may be present. As regards other parts of the central nervous system, however, Bardeen ('03) holds to the opinion that some portion of the nerve cords or of one of them must be present in order that regeneration may occur, while Morgan ('98, '00, '01, p. 44,) believes that regeneration may occur in pieces from the lateral region of the body which contain no part of the longitudinal cords. In an interesting paper dealing



with *Phagocata* and *Dendrocælum*, Lillie ('01) records the fact that while *Phagocata* equals *Planaria* in its regenerative power, the conditions in *Dendrocælum* are widely different. In this form the capacity for regeneration of a head is limited to the anterior third or fourth of the body, pieces from levels posterior to this failing to regenerate. Lillie noted that the pieces of *Dendrocælum* which were incapable of regenerating a head showed a marked difference in reactive power from those in which such regeneration was possible. On the other hand it is known (Loeb, '94, '99, Parker and Burnett, '00) that pieces of *Planaria* deprived of the cephalic ganglia react to stimuli in much the same manner as normal animals. In view of these differences in reactive power Lillie suggests that the stimulation of the normal movements may determine the fate of the undifferentiated mass of new tissue, the head failing to regenerate in the absence of the characteristic stimuli. This suggestion is, I think, an important one.

As Lillie points out, *Dendrocælum* resembles in this respect the earthworm *Allolobophora fætida*, in which, according to Morgan ('97), regeneration of a head does not usually occur posterior to the fifteenth segment. In later work upon this form Morgan ('02) has discovered that the regeneration of the head appears to be closely connected with the presence of an anterior cut surface of the nerve cord, so that if two such surfaces are presented by removing the head and then cutting out a small piece of the nerve cord a short distance posterior to the cut end, a head will regenerate from each of the cut surfaces.

In my previous paper on *Leptoplana* (Child, '04) I suggested that the nervous stimuli in the region of a cut surface may exercise either directly or indirectly some influence upon the growth of new tissue from this region, and, moreover, that after removal of a part they may even be increased in intensity because of the more or less ineffectual attempts of the animal to perform the characteristic movements.

The facts and conclusions cited, together with many others, such as the cases described by Herbst ('96a, '96b, '99) of the substitution of an antenna-like organ for an eye in the absence of the optic ganglion from the eye-stalks of certain decapod crustacea

and the extensive literature of the interesting, although at present somewhat confused question of the relation between the nervous system and the formation and development of the voluntary muscles (Herbst, '01, Neumann, '01, '03, Goldstein,<sup>1</sup> '04) all afford evidence that there is a relation of some sort between the nervous system and the formation of certain structures, at least in some stages of development.

Regarding the nature of this relation various opinions exist. The question as to the "trophic" influence of the nervous system is exceedingly obscure; the formative stimuli of Herbst and others are apparently regarded as entirely distinct from nervous functional stimuli. But that some relation exists between functional stimuli and the development and continued existence of certain structures cannot be doubted.

The occurrence of regeneration in plants, Protozoa and other forms and in stages in which there is no visible differentiation of the nervous system is of course no argument against the influence of the nervous system where it is present. For the development of the nervous system does not add anything to the protoplasm which is fundamentally different from what already exists there. The nervous system is simply a more or less highly differentiated structure which accomplishes the transference and transformation of stimuli, but in its absence some method of transference, however diffuse, must exist in the protoplasm.

The following study of regeneration and other regulative phenomena in relation to the nervous system endeavors to present certain phases of the problem which seem to me important for the form considered.

The figures are diagrammatic but are drawn from careful measurements in nearly all cases. In a number of cases the extent of the intestinal branches is indicated in the figure in a simple manner, no attempt being made to show the actual course of branches in particular individuals. The ganglia are drawn, where present, but the nerve cords are not indicated. The size of the pharynx is shown as exactly as possible: in most cases the

<sup>1</sup> Further references to the literature of this subject may be found in Goldstein's paper.

genital ducts are indicated only by the "genital area" posterior to the pharynx as this was all that could be distinguished with certainty except when the ducts were filled with sexual products.

B. GENERAL CONSIDERATIONS REGARDING THE RELATION  
BETWEEN THE CENTRAL NERVOUS SYSTEM AND  
MORPHOGENESIS.

The existence of a relation between the nervous system and both morphogenesis and the maintenance of form has been established or regarded as probable in various cases, some of which have already been mentioned. Some authors postulate the existence of special nervous "formative stimuli" and "trophic" nervous stimuli have been much discussed. But the relation between the nervous system and morphogenesis is of a problematic character, though the existence of a relation of some sort can scarcely be denied in many cases.

This relation may conceivably be either direct or indirect. In the first case particular nervous stimuli of some sort are to be regarded as constituting in themselves formative factors. In the second case in consequence of certain nervous stimuli a particular part may be subjected to certain conditions which may be the formative factors, though themselves wholly different in character from nervous stimuli. The conditions connected with and resulting from a particular functional activity of a motor organ constitute a good example of the indirect relation. In general the functional activity of a motor organ is determined and controlled more or less completely by the nervous stimuli which affect it and adjoining regions. In consequence of these stimuli it functions more or less perfectly in a particular manner. The functional activity subjects the tissues of the part to a great variety of conditions, physical and chemical, external and internal, which, however, considered as a whole constitute a characteristic complex. Change in the kind or degree of functional activity is of course accompanied by changes in the complex of functional conditions to which the part is subjected. If these conditions play any part in the morphogenesis or form-maintenance a relation between the nervous system and form will appear to exist in such a case, but



upon analysis will be found to be indirect rather than direct. It is to be remembered, however, that even in cases of this kind a direct relation may also exist, *i. e.*, the functional nervous stimuli themselves may conceivably exercise a direct influence of some sort upon the form.

The question as to whether the complex of functional conditions exclusive of nervous stimuli may effect form is undoubtedly to be answered in the affirmative. The existence of a relation between these conditions and form has been established with more or less certainty for various cases by Roux and others. Little attempt has been made, however, to analyze these conditions or analysis has usually not proved very successful. The best examples of so-called functional structures are to be found in the tissues connected with movement. In these structures the arrangement of parts is very closely dependent upon the conditions resulting from use of the organs in a characteristic manner.

In most of the *Turbellaria* as well as in many other forms the whole body is more or less involved in the characteristic movements and thus becomes in a sense a complex motor organ. It is not improbable therefore that the various conditions to which the tissues are subjected in consequence of the characteristic movements are in certain cases important formative factors. I have already shown that such conditions are concerned in form regulation in *Stenostoma* and *Leptoplana* (Child, '02, '03a, '04). As will appear, the description in the following section of the relation between the nervous system and motor activity in *Leptoplana* is a necessary preliminary to the experiments to be presented.

To what extent the functional conditions may constitute formative factors in cases where motor activity is not concerned is a problem regarding which the data are at present few. I am inclined to believe, however, that we shall find form to be essentially functional in very many cases where it is not at present so regarded. Indeed in one sense all organic form is functional.

Among the conditions resulting from functional activity mechanical conditions are important. Their importance has been recognized in connection with the structure of bone, muscle and connective tissue, but I think they are important factors in many



other cases also. The formative effect of these conditions may conceivably be twofold; they may act as stimuli to growth or other changes, *i. e.*, they may exert a "trophic" effect as Triepel and others have pointed out, or they may act in a direct mechanical manner, bringing about a particular arrangement of material. Both of these methods of action are important but the second has been much neglected in the analysis of formative conditions.

The direct mechanical effect of pressure and tension upon the form of parts is, I believe, of great importance and may afford in some cases a simple explanation of phenomena which appear inexplicable from other points of view. A good case in point is the change of form called by Morgan "morphallaxis" in regulating pieces of *Planaria* and other *Turbellaria*. In the case of *Stenostoma* I have shown this change to be primarily mechanical in nature (Child, '02, '03a) and there is no doubt that in other forms the same factors are effective. In the case of *Leptoplana* the effect of mechanical conditions has already been shown in the preceding paper (Child, '04), and will be further considered in the present paper.

But in many cases an indirect relation exists between the nervous system and the mechanical conditions, as in the cases of *Stenostoma* and *Leptoplana* above mentioned, since the mechanical conditions effective here depend upon the use of the parts in a characteristic manner during locomotion. It is thus easy to see how factors, simple in themselves and entirely independent of the nervous system, may apparently stand in relation to it. The same is of course true with regard to other functional conditions as well as the mechanical factors.

Even in cases where a direct relation between the nervous system and form may be shown to exist I see no necessity for assuming the existence of special "formative stimuli" or "trophic stimuli" as distinct from the functional stimuli. Moreover, extreme caution is necessary before concluding that a direct relation exists.

The problem of organic form is undoubtedly the most complex and difficult of all biological problems. I do not think that the suggestions made here tend toward its simplification. The factors of organic form include all the activities of organic substance as

well as the environmental factors in varying degree. Indeed, in most cases, if not in all, we may regard organic form as the visible effect upon the protoplasm of functional activity in the widest sense, occurring in a given environment. But the basis of this functional activity is to be found in the composition of the protoplasm together with environmental factors. I believe this distinction between protoplasmic composition and organic form is important. In general the composition of the protoplasm determines—not form but functional activity of some sort, and in consequence of the internal or external conditions connected with the activity and produced by it form appears. We may say that morphological form is the visible expression of protoplasmic activity in a given environment.

If my experiments succeed in establishing for certain cases certain definite factors in the complex of conditions upon which form depends, something has been gained, especially when we consider the vagueness or the anthropomorphic character of many hypotheses concerning form, and when we remember for instance that Driesch has made certain aspects of the problem of form the basis of his theory of the autonomy of the vital processes, while certain other authors hold that the problem is at present insoluble. If it has proven insoluble thus far I believe it is because of the methods employed rather than the nature of the problem.

### C. EXPERIMENTAL PART.

#### 1. *The Central Nervous System in Relation to Behavior.*

The characteristic movements of the normal animal (Child, '04) are coördinated in such manner that definite characteristic results are obtained: locomotion in a definite direction is possible and the motor reactions to various stimuli possess a definite character. Removal of the cephalic ganglia brings about a marked change in the character of the movements. Pieces without the cephalic ganglia appear at first glance to be in great degree incapable of movement. Careful observation of the pieces shows, however, that they are capable of at least many of the characteristic movements of the species but that those movements are much less powerful and lack coördination.

But another important feature of the movements in the absence of the cephalic ganglia must be noted, viz: that different pieces differ from each other in the degree of coördination, power, and frequency of their movements. Pieces from which the anterior end has been removed by a cut only a short distance posterior to the cephalic ganglia are capable of a somewhat greater degree of activity than those from which the anterior half or two-thirds of the body has been removed. In general it appears that the greater the remaining portion of the central nervous system the more complete the activity.

We may consider first the case of a specimen from which the anterior end has been removed by a transverse cut two or three millimeters posterior to the cephalic ganglia. Such a piece is capable of locomotion but the advance is very slow and uniform. In my account of the normal movements (Child, '04) I called attention to the fact that locomotion in *Leptoplana* is accomplished both by means of cilia and by muscular contraction, parts of the margin being extended and attached to the substratum and then undergoing contraction, thus dragging the body forward. The muscular factor is especially conspicuous after strong stimulation. In the specimen deprived of the cephalic ganglia, however, progression is accomplished largely by means of cilia, hence the slow, uniform, gliding character of the movement. The specimen is apparently capable of performing all the muscular movements necessary for muscular locomotion but they appear to lack perfect coördination. Occasionally the piece seems to succeed in using its muscles in some degree effectually, but it is probable that these instances are simply due to chance coincidence of particular muscular contractions. As the piece is more and more strongly stimulated the muscular contractions become more and more violent, although not coördinated, until finally the whole piece is involved in convulsive movements during which it may roll up and unroll or twist and squirm about, often turning over with ventral surface uppermost.

Use of the posterior margins and posterior end of the body as organs of attachment occurs to some extent in these pieces. As the piece glides over the substratum parts of these regions can be



seen to attach and free themselves in the characteristic manner, though here the muscular play of the margins is much less marked. The piece as a whole adheres much less closely to the substratum, however, than the normal animal. It is not at all difficult to detach these pieces by means of a current of water from a pipette, while the normal animal adheres so closely that detachment by this method is often almost impossible.

According to these observations pieces without the cephalic ganglia show both a quantitative and qualitative difference from normal animals as regards motor activities. All motor activities appear to be less intense than under ordinary conditions and the imperfect coördination in muscular movements alters the character of the movements very greatly.

In these pieces the margins of the head, apparently the chief tactile organs, are of course absent and other parts of the body are less sensitive than these. Reaction to tactile stimulation of the lateral and other regions of the body is, however, less intense and definite than in pieces containing the cephalic ganglia. The eyes are also absent in these pieces and there is no marked reaction to light, though in a few cases, I thought I could observe some slight reaction (compare Parker and Burnett, '00).

Individual differences in the behavior of pieces without ganglia are often observed even where the cuts removing the head were at the same level. Some pieces seem capable of more complete coördination than others, as is clearly seen, for example, by the rapidity with which they right themselves. These individual differences are of most frequent occurrence when the cut is not far from the ganglia and may be due to slight differences in level of the cut, one piece retaining some parts of the nervous system absent in others. Occasionally, however, they occur when the cut was some distance posterior to the ganglia, and in such cases must probably be ascribed to some structural or physiological difference of which at present we know little. The fact of the existence of such differences is however of interest as probably indicating the existence of marked variations of some kind in the nervous system.

There seems to be some degree of correlation between size and



the ability to perform coördinated movements in these pieces deprived of ganglia. Of two pieces with anterior ends at the same level the longer seems to show a slightly greater degree of coördination; it is sometimes able to advance more rapidly than the shorter piece and in general appears to be less completely helpless. The piece which has lost the greater posterior part of its body does not make up for this loss by greater use of the regenerating part to any such extent as does the piece with ganglia, but is simply more helpless than the piece which has lost only a small part. Exceptions are frequent but I think that a real difference does exist. It is necessary to distinguish two factors here, viz: the power of motor activity in general, *i. e.*, the power of performing movements of any kind, and the power of coördinate functional activity. The smaller pieces usually appear to be more active than the larger but their activity seems to be less perfectly coördinated and so less effective as regards locomotion, etc. I am inclined to believe that the greater activity of the smaller pieces is connected with the loss of a large part of the body as is the case in similar pieces with ganglia, while on the other hand the lack of ability to coördinate is probably due to the small portion of the central nervous system present.

The question as to whether the pieces deprived of the cephalic ganglia retain the power of "spontaneous" movement is somewhat difficult to answer, since no sharp distinction can be made between spontaneous movements so-called and complex series of movements following particular stimuli; indeed in my opinion no distinction save one of degree exists. The pieces without cephalic ganglia are certainly much less active than normal animals, react more slowly and less strongly to stimuli and, as has been mentioned, are unable to a large extent to coördinate their muscular movements. But even when apparently undisturbed such pieces are often found moving slowly about and performing indefinite muscular movements similar in character to those of normal animals but not correlated. I am inclined to believe that the loss of the cephalic ganglia means essentially the loss of the connections with the principal sense organs, *i. e.*, the organs for the reception of stimuli, and the loss of a part of the more or less complex con-

ducting paths. This being the case we should expect to find less power of reaction to stimuli, less complexity and a lower degree of correlation in the movements. These are exactly the conditions that we do find.

It is, I think, desirable to avoid the use of the word "spontaneous" in this connection since the difference between spontaneous and non-spontaneous movements seems to be merely one of degree of complexity and correlation or coördination. Removal of the principal paths by which stimuli enter and a part of the structures which connect these paths with other parts of the nervous system must reduce the complexity of structure and consequently of the visible activities dependent upon this structure.

Among these observations the most important point for the present consideration is the presence of the power of progressive locomotion in some degree in pieces deprived of the ganglia.

Loeb ('94, '99) found that in the case of *Thysanozoon* loss of the power of progressive locomotion resulted from the removal of the cephalic ganglion. This is certainly not the case in *Leptoplana*, and indeed experiments of my own upon *Thysanozoon* led me to the conclusion that even here the pieces without the ganglia still possessed some slight power of locomotion, though much less than that of the normal animal. In both *Thysanozoon* and *Leptoplana* these pieces are capable of righting themselves after being turned over, but the change in position is much less rapid than in normal animals and frequently is accomplished only after repeated attempts, or in some cases does not succeed at all, and the piece gradually becomes quiet.

One other point of considerable interest must be considered. In *Leptoplana* I observed a marked difference in the power of locomotion and of coördination in general in pieces cut at different levels, the activity decreasing as the portion of the body removed with the cephalic ganglia increased. If, for instance, an individual was cut transversely two or three millimeters posterior to the ganglia the posterior piece was much more active and was capable of more rapid locomotion and more perfect coördination than a posterior piece obtained by a cut posterior to the middle of the body. In general the greater the distance between the cut and

the ganglia the less the activity and the more irregular and imperfect are the movements. The difference between a piece obtained by a cut just posterior to the ganglia and one from the region posterior to the pharynx is striking. The latter scarcely reacts at all to stimuli, is almost wholly incapable of progressive locomotion and rarely succeeds in righting itself—though this last may be due in part to the fact that such pieces are necessarily short—while the activity of the former is much greater in all respects though far below that of the normal animal.

So far as I am aware the case of *Dendrocælum* mentioned by Lillie ('01) is the only one in which observations of this kind have been made. In *Dendrocælum* Lillie found that posterior pieces obtained by section in the anterior third or fourth of the animal reacted to light like normal animals though more slowly, while pieces from levels posterior to this did not react. He does not mention any degree of reactive power corresponding to difference of level of the cut in pieces capable of reacting, but since other *Turbellaria* which I have observed resemble *Leptoplana* more or less closely, I am inclined to think that such a difference may possibly be present, though I have not had the opportunity of examining *Dendrocælum* in sufficient numbers to decide this point.

The differences in pieces from different levels are not due simply to differences in size, for a short piece from the region just posterior to the cephalic ganglia is much more active than a piece of the same size from the posterior region. It is apparently not simply the amount of nerve tissue present that determines the degree of motor activity, but rather the quality of this tissue which differs in different regions. With our present knowledge of the nervous system only vague surmises as to the nature of this difference are possible. It may be, at least in part, a difference in structural complexity, or the difference in the quantity of energy transformed by the stimuli or it may be something different from either of these: as a matter of fact the nerve cords in the *Turbellaria* diminish in size toward the posterior end of the body. But the fact that a difference exists is important. My observations also indicate that the case is somewhat similar as regards the ceph-



alic ganglia: in general the motor activity falls further and further below the normal as the portion of the ganglia removed or injured increases. It is often difficult with the present technique of operation upon these forms to determine the extent of injury to the small ganglia, but notwithstanding this difficulty my observations indicate very clearly that a relation exists between coördinated motor activity and the amount of ganglionic tissue present. When the cut passes through the middle of the ganglia both pieces separated behave essentially like normal animals, but when less than half of the ganglionic tissue remains intact the piece behaves much like specimens without ganglia and if the portions of the ganglia remaining are very small there is almost no motor activity except the ciliary movement, unless the piece is strongly stimulated. Since the ganglia are small and the difficulty of making a section in them at exactly the level desired is great, and since it is often difficult to determine after section just what parts of the ganglia remain, the results of these experiments are not exact. But the fact that the two pieces of an individual separated by a cut through the middle of the ganglia both behave like normal animals shows that the removal of half of the ganglionic tissue does not affect the behavior appreciably. Moreover, it makes no difference in such cases whether the cut is longitudinal or otherwise. Anterior and posterior halves and right and left halves of the ganglia seem to be essentially alike in this respect.

Pieces from the region anterior to the ganglia show almost no motor activity except that of the cilia, which continue to beat, and some degree of contraction after strong stimulation. Such pieces die in the course of two or three days.

These relations between the various regions of the nerve cords and the cephalic ganglia and coördinated motor activity will be illustrated in the consideration of individual cases. The fact of the relation is of interest and indicates, in my opinion, that co-ordination is connected in these forms rather with a certain extent and structural complexity than with certain definite organs or centers. Certainly the cephalic ganglia are more important for motor activity and coördination than the other portions of the nervous system, but it is possible that their connection with the



chief sense organs, *i. e.*, the paths by which more or less definitely localized stimuli enter the nervous system, is the primary factor in their predominance.

With regard to the existence of "centers" in the nervous system I agree essentially with Loeb ('99) and I think the relations above described support this view. Coördinated movements are the result of series of interrelations and exist after mutilation in the degree in which the interrelations remain intact or are reëstablished.

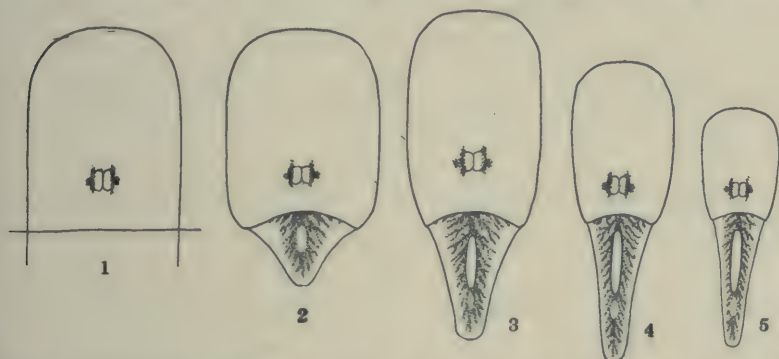
The case of *Leptoplana* as cited affords strong support to the view that the difference between "spontaneous" and "non-spontaneous" motor-activity is simply one of degree. Moreover, it is impossible to say that one part of the central nervous system in *Leptoplana* is necessarily connected with coördinated movement while another is not. It is rather the amount of nervous tissue—in all probability the completeness of the system of connections of parts—than the presence of any one portion which determines the results.

## 2. *The Relation Between the Central Nervous System and Posterior Regeneration.*

From Schultz's ('02) account of regeneration in *Leptoplana atomata*, it is evident that there is but little difference as regards regeneration between this species and *L. tremellaris*, but in the only case in which Schultz and I are really concerned with the same problem our interpretations of the facts differ widely.

As regards the limits of regeneration in *Leptoplana* a brief preliminary statement will suffice here. Posterior regeneration from a cut surface is qualitatively complete at all levels posterior to the cephalic ganglia whether these are present or not and anterior regeneration is complete only when the ganglia are present at least in large part, *i. e.*, only anterior to them. In other words, regeneration of a head is impossible in the absence of the cephalic ganglia but posterior regeneration occurs whether they are present or not. In the absence of food the size of the new part is never as great as that of the part removed, but this is not of great importance.

The course of regeneration in the posterior direction from a level between the cephalic ganglia and the pharynx is illustrated in Figs. 1-5. On these figures the organs are indicated in a somewhat diagrammatic manner. The intestine is not drawn in the old parts, but the general distribution of its branches is indicated in the regenerated parts. Fig. 1 indicates the level of the cut and the shape of the anterior end before section. After section the cut surface contracts and becomes concave posteriorly, and within two or three days new unpigmented tissue appears. In Fig. 2 the condition of the piece ten days after section is indicated. An outgrowth of new tissue tapering posteriorly is present, into which intestinal branches extend from the old part—and it may be mentioned in passing that the intestine in regenerated



areas apparently always arises in connection with the old parts present. In the median line is a small ill-defined area which represents the developing pharynx. Fig. 3, sixteen days after section, shows a more advanced condition. The regenerated area is longer and the pharynx is distinct. From this time on a marked decrease in size occurs but the old part is much more affected than the new, as is indicated by Fig. 4 twenty-seven days after section. Here the new and old parts are of equal length, the new being longer, though perhaps not containing more material than in Fig. 3, and the old shorter. The pharynx has increased in size and beyond it a small clear area, which may be called the genital area, indicates that regeneration of the genital ducts is taking place. Fig. 5 shows a stage fifty-one days after

section. The old parts have continued to decrease in size more rapidly than the new, but otherwise there is little difference between this and the preceding stage. This piece remained alive during another month, the change in relative size of old and new parts continuing, together with reduction in size of the whole.

The history of this piece is typical for posterior regeneration from this level of the body. Differences in the amount of regeneration occur in different individuals, but in all cases regeneration may be said to be qualitatively complete in that the characteristic organs of the part removed are regenerated. As the size of the part removed decreases so in general does the amount of regeneration. The significance of this fact will be discussed more fully later. When parts of the pharynx or genital ducts are present the regeneration apparently always begins from the old part, but when such organs are wholly removed they are formed anew. In general the level at which regeneration occurs, the presence or absence of food, and individual differences affect the regeneration quantitatively but not qualitatively.

a. Experiments on the Relation between the Cephalic Ganglia and Posterior Regeneration at Various Levels behind the Head.

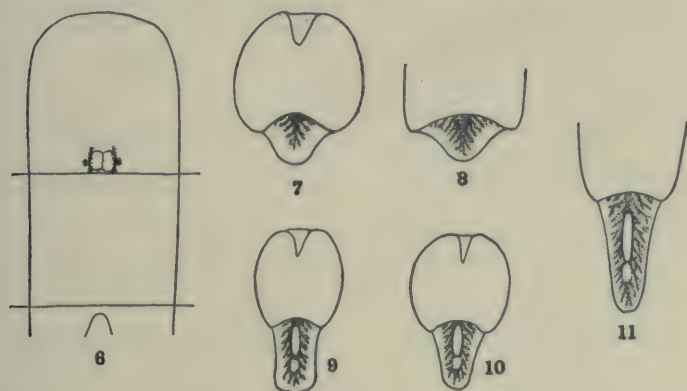
Mention was made above of the fact that *Leptoplana* is not capable in any case of regenerating a head in the absence of the cephalic ganglia. This apparent dependence of anterior regeneration upon the cephalic ganglia has been established for a number of forms but the question as to the relation between the cephalic ganglia and posterior regeneration in the *Turbellaria* has received little attention. In order to examine this problem I prepared series of pieces as follows: a certain number of specimens of as nearly as possible the same size were cut at a given level and from half of these the cerebral ganglia were removed by a transverse cut just posterior to them; the regeneration of the two sets was then compared at stated intervals with respect to rapidity, amount, and quality of regeneration and the form of the new part. In several cases also series prepared for other purposes proved of value in this connection and could be compared with other pieces cut at



the same level which were not originally intended as controls for them. These experiments were performed during the winter when the temperature of the water was much lower than in summer and the total amount of regeneration in the various cases is less than in summer experiments. In all cases the pieces were kept until regeneration ceased, in order that comparison of the total regeneration might be made.

*Series 73.* Six pieces, each representing the region of the body between the cephalic ganglia and the pharynx, were obtained by the two transverse cuts indicated in Fig. 6.

*Series 82.* Five pieces were obtained by transverse cuts just anterior to the pharynx (the lower line in Fig. 6) but the head



and cephalic ganglia were left intact. This series was not originally intended as a control for Series 73 consequently the intervals between examinations are somewhat different though not enough to prevent comparison.

Fig. 7 shows the condition of the pieces of Series 73 eighteen days after section and Fig. 8 the posterior ends of the pieces of Series 82 fourteen days after section. In Series 73 the contraction of the cut surface is greater, the new tissue contains fewer intestinal branches, and the amount of the new tissue is somewhat less than in Series 82.

The different pieces of each series were so closely similar that these two will serve as examples.



In Figs. 9 and 10 the condition of the pieces of Series 73 thirty-eight days after section is indicated. In one of the pieces the new tissue showed the tapering form of Fig. 10, the other pieces resembling Fig. 9. The former piece was capable of more rapid locomotion than the others. In all the pharynx and genital area are visible and an axial intestine with short branches extends down the middle of the pieces.

The condition of the pieces of Series 82 thirty-four days after section is indicated in Fig. 11. Different pieces differed slightly as regards the length of the new tissue, but other differences were not observed. Pharynx and genital area were present and the new tissue was well-filled with intestinal branches.

The pieces of Series 82 differ markedly, however, from those of Series 73 in that the amount of regeneration is much greater in the Series 82, where the cephalic ganglia are present. Moreover, comparison of the Figs. 9 and 10 with Fig. 11 shows that the pharynx is longer and the intestinal branches much more abundant in Series 82.

After this time there was no further advance in regeneration. The pieces of both series had already begun to decrease in size and continued to do so, but the decrease was somewhat more rapid in Series 82 than in Series 73.

In these two series the differences seem to be wholly quantitative. The pieces in which the cephalic ganglia are intact regenerate more rapidly, at least in the later stages; the amount of new tissue formed is greater; the pharynx is larger; and the intestinal branches are more numerous.

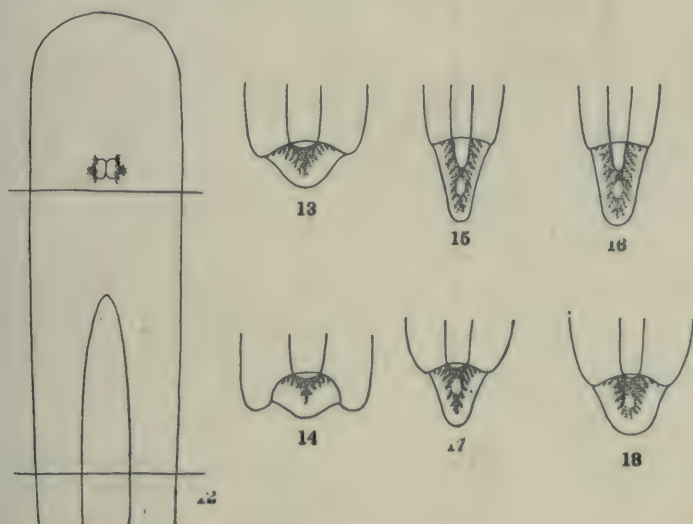
*Series 78.* Five specimens were cut transversely through the middle of the pharynx (Fig. 12) the anterior part with head and cephalic ganglia intact being used.

*Series 79.* Five specimens were cut at the same level as in Series 78 but in these the head was removed by a second cut just posterior to the ganglia (Fig. 12).

Figs. 13 and 14 indicate the condition of the posterior ends in the two series fourteen days after section. In Fig. 14 (Series 79, without cephalic ganglia) the contraction of the cut surface is greater and the new tissue contains fewer intestinal branches

than in Fig. 13 (Series 78, ganglia present). There is no marked difference in the amount of regeneration.

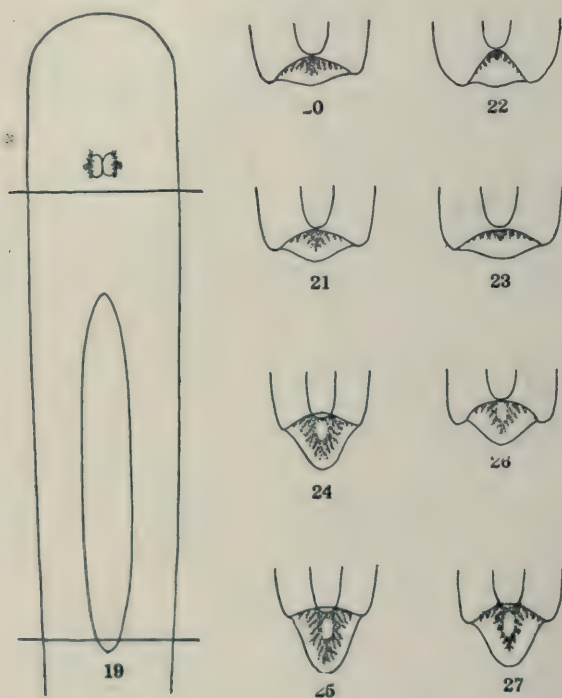
Thirty-four days after section the pieces of Series 78 have attained the condition represented in Figs. 15 and 16, and Figs. 17 and 18 represent the condition of Series 79. Here, as in the preceding case there is a marked difference between the two series. In the series containing the cephalic ganglia, the amount of regeneration is greater, the posterior portion of the new pharynx has regenerated in the new tissue to a much larger extent, and intestinal branches fill the new tissue much more completely. Later stages afford no additional features of interest.



*Series 80 and 81.* In these two series the level from which posterior regeneration occurred was at the posterior end of the pharynx (Fig. 19). Series 80 consisted of five pieces with head and cephalic ganglia intact and Series 81 of five pieces from which the head and ganglia had been removed (Fig. 19).

Figs. 20 and 21 represent the condition of Series 80 fourteen days after section and Figs. 22 and 23 of Series 81 after the same interval. There is little difference between the two series at this stage except that in Series 80 the intestinal branches have penetrated further into the new tissue.

Figs. 24 and 25 show the two extremes of Series 80 thirty-four days after section and Figs. 26 and 27 those of Series 81 after the same interval. Comparing the two pieces showing least regeneration in the two series (Figs. 24 and 26) the amount of regeneration in the piece from Series 80 (Fig. 24) is the greater (Fig. 26), and a similar difference, though less marked, occurs in the pieces showing the maximum regeneration (Figs. 25 and 27).



\* Moreover, three pieces of Series 80 were essentially like Fig. 25, while only one in Series 81 was like Fig. 27. As regards the intestinal branches all pieces of Series 80 are in advance of Series 81. No further changes occurred in later stages.

Thus in these series as in the others above described the pieces containing the cephalic ganglia are quantitatively in advance of those without ganglia.

*b. Discussion of the Experiments.*

These three pairs of experiments at three different levels of the body all afford similar results. In all the series containing the ganglia regeneration is quantitatively more complete than in those where the ganglia are absent. Moreover the difference is greatest between Series 73 and 82, is less but still considerable between Series 78 and 79, and is only slight between Series 80 and 81, *i. e.*, the difference decreases with increasing distance of the cut surface from the anterior end. And finally, there is in general a decrease in the absolute amount of regeneration in all series with approach of the cut surface to the posterior end.

In order to forestall the possible objection that differences in thickness in the dorso-ventral dimensions of the new tissue have not been taken into account it should be said that frequent observations upon this point showed no marked difference in thickness, though usually the new tissue in pieces without ganglia was not as thick as in the others, probably because the cut surface undergoes greater contraction dorso-ventrally as well as in other directions in such pieces without ganglia.

In all three cases the pieces without ganglia are smaller than the others, since the whole head was removed with the ganglia, but only in the first case (Series 73 and 82) is the difference in size very great; here the pieces without ganglia are only about half the size of the others. As a matter of fact, however, the differences in size do not appreciably affect the result, as numerous experiments have shown me. Other things being equal a smaller piece becomes exhausted and dies sooner than a larger piece, but except in the case of minute pieces both live several months. Moreover, in the above experiments, the smallest pieces without ganglia (Series 73, Figs. 9 and 10) show more regeneration than either of the other series without ganglia (Series 79, Figs. 17 and 18; Series 81, Figs. 26 and 27) and the same is true of the pieces with ganglia. The long pieces with ganglia of Series 80 (Figs. 24 and 25) show less regeneration than do the small pieces without ganglia of Series 73 (Figs. 9 and 10). It is evident that the differences in size of the pieces cannot account for the results of these experiments.



Is it then possible that certain "formative stimuli" which affect posterior regeneration are connected in some manner with the presence of the cephalic ganglia? If such exist they certainly do not concern particular organs for regeneration of all the organs characteristic of the part removed takes place in the absence of the ganglia, though these organs are of smaller size or less complex in arrangement than when the ganglia are present, as for instance the pharynx and intestinal branches. Moreover, the differences between the two groups differ to a considerable extent with the region from which regeneration takes place. It is clear that we cannot suppose that any particular "formative stimuli" are connected with the presence of the ganglia. We may, however, take the position that all stimuli to growth are more powerful when the ganglia are present and so bring about a greater amount of regeneration.

But can we proceed a step further and reach any conclusions as to the character of these stimuli? I believe that this is possible, and moreover, that these experiments afford valuable data for the interpretation of certain regulative processes in these forms.

But first it is necessary to recall what was said in an earlier section (pp. 470 and 471) regarding the behavior of the pieces deprived of the cephalic ganglia. The imperfect coördination of movement and the less intense and complex activity are the chief points in which these pieces differ from those in which the ganglia are present. Locomotion is slow and chiefly ciliary, all movements are weaker and there is in general much less movement of all kinds in the absence of the ganglia. These facts are of great importance for the consideration of this problem as the following paragraphs will show.

The chief points for consideration are as follows: first, what is the reason for the difference in regenerative power between pieces with and those without ganglia? Second, why is this difference most marked in the anterior regions of the body? Third, why does it appear chiefly during the later stages of regeneration and only to a slight extent in the earlier? Fourth, why does the form of the regenerated part as a whole differ in the two cases? Fifth,

why are certain structures formed in the new tissue of smaller size or of less complexity in the absence of the ganglia? It is hoped that the following discussion of these points may throw some light upon the problem and afford some hints for future investigation.

The only satisfactory answer to the above questions is to be found in the differences in functional activity of the parts in the two pieces. In the pieces with cephalic ganglia, the locomotion being much more rapid and all movements more intense, the posterior parts including the new tissue are used to a much greater extent than in the pieces without ganglia. This greater functional activity comprises many elements, attachment of the margins and posterior end to the substratum and consequent tension upon the parts; the constantly changing but characteristic mechanical conditions to which the parts are subjected in consequence of the coördinated muscular activity and the pressure of the intestinal contents and perhaps of other internal fluids resulting from the movements; and the motor stimuli which may possibly influence growth—these are some of the conditions which accompany the motor activity of a given part, many of which in my opinion may be formative factors. All of these conditions are present in much greater degree in the pieces with intact ganglia, and since the posterior part of the body has been removed, the region of the cut surface and the new tissue as it appears must be especially affected by them for these parts so far as function is concerned supply the place of the parts removed. For example in a case where the greater part of the body has been removed as in Series 73 and 82, the region of the cut surface and the new tissue arising from it are used by the animal, or at least the attempt is made to use them, as the part removed would be used if it were present. The greater the degree of functional activity affecting these parts the greater the stimuli to growth and the more powerful the mechanical factors which assist in arranging the new material or perhaps themselves stimulate growth. Thus the first of the above questions finds its answer in the fact that the functional activity of the regenerating region is greater in the pieces with ganglia than in those without. I think no one who com-

compares the behavior of two such pieces can fail to be convinced of this fact and of its importance in connection with regeneration.

The second question—why the difference in the amount of regeneration in the two sets of pieces is most marked in the anterior regions of the body follows directly from the answer to the first. It is evident that when a large part of the body has been removed the regenerating tissue which arises in its place must be subjected to a much greater degree of functional activity than when it supplies the place of only a small part. For example if the whole body posterior to the anterior end of the pharynx be removed as in Series 73 and 82 the new tissue which grows out from the cut surface is the functional representative of the part removed. In locomotion it serves not only as a posterior end for attachment but also takes the place of the lateral margins of the long piece removed. There can be little doubt that in such a case all conditions correlated with functional activity must be present in much greater degree than in new tissue which grows out from a cut surface near the posterior end and which supplies the place of only a small portion of the body. If these conditions constitute factors in regeneration posterior regeneration must decrease in amount with the approach of the cut surface to the posterior end. The three sets of pieces with ganglia, Series 82 (Fig. 11), Series 78 (Figs. 15 and 16), and Series 80 (Figs. 24 and 25) show this difference in the amount of regeneration very clearly. In the pieces without ganglia, however (Series 73, Figs. 9 and 10; Series 79, Figs. 17 and 18; Series 81, Figs. 26 and 27), the difference is much less marked. In the first series in which the cut surface was near the anterior end (Figs. 9 and 10) the amount of regeneration is slightly greater than in the other two series (Figs. 17 and 18 and 26 and 27) but between these two there is little difference.

It is necessary here to recall what was said in an earlier section (pp. 471 and 472) on the relation between size and coördination in pieces deprived of ganglia. Of two pieces with anterior ends at the same level the larger piece seems to be slightly less helpless than the smaller piece though the latter is often more active. In view of these facts we cannot expect to find any such difference between



the pieces of Series 73 (Figs. 9 and 10) and those of Series 81 (Figs. 26 and 27) as between Series 82 (Fig. 11) and Series 80 (Figs. 24 and 25) in regard to the functional activity of the new tissue. The greater activity of the smaller pieces without ganglia is, to a certain extent, counterbalanced by their more imperfect coördination. Numerous comparative observations were made upon the pieces of the three series in order to determine if possible whether actual differences in motor activity did occur, and I concluded that in the pieces of Series 73 (Figs. 9 and 10) the new parts were somewhat more active than in the other two series, though the coördination of movements appeared to be more imperfect. Thus it is evident that these pieces considered by themselves afford few data of importance for or against the present view, since the differences in activity are at best slight. But the fact that the difference in the amount of regeneration at different levels is much less marked in pieces without ganglia than in those with ganglia is what might be expected according to the views above expressed, since the differences in motor activity in the different cases are certainly much less in the former than in the latter series.

The consideration of the third question is next in order, viz: why the difference in the amount of regeneration between pieces with and those without ganglia appears chiefly during the later stages of regeneration and only to a slight extent or not at all during the earlier stages. Comparison of Figs. 8, 13 and 20 and 21 (pieces with ganglia) with Figs. 7, 14 and 22 and 23 (pieces without ganglia) shows that during the first two weeks the amount of regeneration does not differ greatly in the two sets. In most cases the amount of new tissue seems to be slightly greater in the pieces with ganglia, but the difference is not very marked.

It is not possible at present to reach definite conclusions, but certain probable reasons for this condition suggest themselves. In the first place it seems probable that the first outgrowth of new tissue from a cut surface in cases of this kind is determined by factors different from those which determine the later regeneration. Indeed it is by no means certain as yet how far this apparent formation of new tissue is due to actual proliferation and how far



to change in position of cells from the old part. The removal of a part leaves a more or less widely open wound and the soft tissues of the body may gradually migrate or flow out in consequence of altered conditions of surface tension or capillarity, or may be forced out by internal pressure in consequence of muscular contractions in the old part. The rounded form of this new tissue suggests the possibility that surface tension may play a part in its formation. At the same time it is probable and indeed certain in many cases that rapid proliferation of the cells near the cut surface does occur. This multiplication has been ascribed in a general and somewhat vague manner to the altered conditions at the cut surface, doubtless a correct conclusion as far as it goes. The possibility that altered conditions of surface tension and pressure resulting from the removal of the part which originally adjoined the cut surface may themselves bring about multiplication has, however, received little attention, although various experimenters have shown that cell division is influenced by changes in these conditions. Various other physical and chemical factors resulting from the injury may also be concerned in this process, but the point to which I desire to call especial attention is that the appearance of new tissue from the cut surface is not primarily a regeneration of anything in particular, but may be largely a flowing out of the soft viscid contents of the body in consequence of reduced pressure in this direction or altered conditions of surface tension accompanied by a more or less rapid multiplication of cells which is itself the result of the actual conditions. In short the factors concerned are local and are mostly present whether the parts are used in a particular manner or not. The relative amounts of transposition and of proliferation doubtless differ widely in different species according to their consistency and reactive capacity.

If we admit that the first appearance of "new tissue" from the cut surface is determined by these relatively simple local factors there is no obvious reason why in a given species the amount of this new tissue formed from a cut surface at a given level in a given time should not be approximately the same in different individuals, whether the ganglia are present or not. The only way in which the presence or absence of the ganglia might affect the result lies,

so far as I can see, in the frequency of muscular contraction and the consequent internal pressure upon the regions adjoining the cut; it is possible that more frequent contraction and movement might force the tissues out through the wound more rapidly, though this is not a factor of great importance in any case.

In the cases under consideration at present I think we may regard the new tissue present two weeks after section as representing to a large extent this first stage. At this time the new parts are functional in movement to only a very slight degree and may be regarded as practically undifferentiated outgrowths. It is possible that the slightly greater amount of this tissue in the pieces with ganglia may be due to the more frequent movements which have so to speak forced more material out through the wound, or it may be that we have here the beginnings of the difference which is more marked in later stages. In earlier stages than those figured the differences were, as might be expected, even less marked.

For the purpose of analysis we may consider the stage of differentiation as following the first indifferent stage which we have considered. As a matter of fact the two overlap in varying degree and manner according to circumstances. The fate of the new material must be regarded as depending essentially upon its relations to the old parts, or in the words of Driesch, its fate is a function of its position, since these relations determine what stimuli it receives and to what conditions external and internal, it is subjected. Under ordinary circumstances the new part supplies functionally the place of the part removed though very imperfectly at first; or, in other words, the animal or piece attempts to use it as it would use the part removed if this were present. I am inclined to believe that this "attempt at use" is an important factor if not the most important in determining what the new part shall become. But from the time when the use of the part begins, or let us say, when functional stimuli reach it, it is subjected to conditions differing widely from those of the first stage. By these conditions the "indifferent" material is moulded into its definitive form and structure. The influence of the conditions connected with locomotion on the form of regenerating posterior regions has

been considered by me in several papers (Child, '02, '03a, '04). It is these same conditions which we have to consider in the present case, and the present question becomes from this stage on identical with the first, which has already been discussed.

According to this view then regeneration in the earlier stages is about the same in pieces with and those without ganglia because it depends to a large extent upon local factors connected with the absence of the part removed and the presence of the cut surface while in later stages the special functional conditions connected with the use of the part in a characteristic manner, determined essentially by its position in relation to the whole, constitute the important factors in determining both the amount of regeneration and the structural differentiation.

The fourth question has special reference to the general form or outline of the regenerated part which is as a rule more slender and tapering in the pieces with ganglia than in those without, the difference being greatest when the cut surface is near the anterior end.

The answer to this question is simple. I believe that the difference in form is due primarily to the tension in the direction of the longitudinal axis exerted upon these parts in consequence of the use of the posterior end during locomotion as an organ of attachment. In *Leptoplana* both the posterior end and the lateral margins are employed for attachment to the substratum and are in consequence subjected to tension as the animal moves forward holding by one part or another of this region. Brief examination of creeping specimens is sufficient to show that these conditions exist. In pieces containing the ganglia the use of these parts in this manner is much more frequent and the tension is much greater since locomotion is much more rapid than in pieces without ganglia. Undoubtedly these conditions play a part in the arrangement of the physically plastic new material, as was very clearly shown in the preceding paper of this series (Child, '04) and it is not improbable that they also serve as stimuli to growth. The form of the new part in Figs. 11, 15, 16, and especially in Figs. 2-5 (summer experiments) is very evidently a form resulting from mechanical tension exerted chiefly at the posterior end.



It is difficult to understand otherwise why the margins of this region are concave instead of convex or straight.

But the form differs according to the level from which regeneration occurs (compare Figs. 11, 15 and 16, 24 and 25). The tapering outline with concave margins is most marked when regeneration takes place from a level near the anterior end. This difference is of course connected with the fact that the amount of regeneration is greater in anterior regions but that difference does not explain why the new tail should be more slender in the one case than in the other. Evidently this difference in form of the new part at different levels is mechanical. In the first place, when the regenerating part represents a large part or all of the region of the body used for attachment it performs the functions of that part and is subjected to the tensions resulting from this function. When, however, as in Figs. 24 and 25, it represents only the posterior region of the part used for attachment the lateral margins of the old portion anterior to it perform in large degree the function of attachment and hence the regenerating part is subjected only to a relatively slight degree of tension. Therefore, it is less elongated, less concave laterally and more blunt posteriorly.

In the pieces without ganglia these differences are naturally much less marked since the tension upon the parts resulting from locomotion is relatively slight in all cases. But even here there is a difference, at least between Series 73 and the others (compare Figs. 9 and 10, Series 73, with Figs. 17 and 18, Series 79, and Figs. 26 and 27, Series 81). The peculiar form of the regenerating part in Fig. 9 seems to be due to the fact that this piece used one part of the margin as often as another for attachment, whereas usually the median posterior region is used more than other parts. The form of the regenerating part shown in this figure is common in such pieces and may, I think, be regarded as due primarily to lack of coördination; any part of the margin which happens to come into close contact with the substratum or which is stimulated in any other way becomes attached and consequently the part does not taper posteriorly. The piece shown in Fig. 10 was able to progress more rapidly and in general showed a higher degree of coördination than the other. The difference in the



form of the new part in these two pieces corresponds very closely with the differences in its use.

The last of the points to be considered in connection with these pieces is that which concerns the size and complexity of the characteristic organs of the regenerated part, especially the pharynx and the intestine. In the Series 73 and 82, in which regeneration occurred from a level near the anterior end, the pharynx is much larger and the intestinal branches are much more fully regenerated in the pieces with ganglia (Fig. 11) than in these without (Figs. 9 and 10).

In the second pair of series, in which regeneration occurred from a level near the middle of the body, the regeneration of the posterior end of the pharynx is much more complete in the pieces with ganglia (Figs. 15 and 16) than in those without (Figs. 17 and 18), and here again we find a difference in the extent of the intestinal branches similar to that in the preceding sets.

In the third pair of series, in which regeneration occurred from a level at the posterior end of the pharynx, regeneration of the pharynx does not take place but there is the same difference in the number and extent of the intestinal branches that has been observed in the other cases (compare Figs. 24 and 25 with Figs. 26 and 27).

As regards the pharynx the difference in size in the two sets is doubtless an expression of the proportionality characteristic of regenerating parts; the greater the amount of regeneration the larger the pharynx. But why does such a proportionality exist? This is a difficult question to answer and Driesch has even asserted that it cannot be answered in physico-chemical terms. It is true that we are at present unable to analyze the factors concerned, but I see no reason for assuming an autonomistic or vitalistic principle. The conditions of the case seem to me to be somewhat as follows: The regenerating part represents a certain region of the body and its relations to the old part determine that it shall function in a characteristic manner, *i. e.*, in the manner of the part which it represents. Consequently the area affected by particular functional stimuli will be more or less nearly proportional to the size of the regenerated portion.

Or we may put the case in a somewhat different form: admitting that the total amount of regeneration is proportional to the degree in which the conditions for regeneration are present, each part of the regenerating portion attains a certain size and form which represent in a way the proportionality between the stimuli affecting it and those affecting other parts. If, as I have suggested, these stimuli are at least in part the functional stimuli, we may say that the size of the pharynx in a regenerating part is dependent upon the size of the part and upon the relation between functional conditions affecting the pharynx and those affecting other regions.

In short, the difference in size of the pharynx in the pieces with and those without ganglia is simply a particular expression of the factors which determine the difference in the amount of regeneration in the two cases, viz: the functional conditions. If, as Bardeen ('01, '03) supposes, the pressure of the intestinal contents is a factor in the formation of the pharynx, it is evident that this factor must be present in much greater degree in the pieces with ganglia since the movements and muscular contractions of the various parts which force the intestinal contents out of the branches toward the central parts are much more frequent and intense in these pieces than in the others. At any rate it is evident that the regenerating part is functionally active in much greater degree in the presence of the ganglia, and since I believe that the various conditions connected with functional activity are important factors in the production and maintenance of organic form, the difference in size of the regenerated pharynx in the two sets is in full agreement with the other facts already discussed.

The extent to which the intestinal branches regenerate also differs in the pieces under consideration. Examination of the figures will show that in every case, even in the earlier stages, the regeneration of the intestinal branches is more advanced in the pieces with ganglia. Compare Figs. 8 and 11 (ganglia present) with Figs. 7, 9 and 10 (ganglia absent), Figs. 13, 15 and 16 (ganglia present) with Figs. 14, 17 and 18 (ganglia absent), and Figs. 20, 21, 24, 25 (ganglia present) with Figs. 22, 23, 26, 27 (ganglia absent).

The intestinal branches appear to rise in all cases from the cut

ends of parts of the old intestine and extend from these regions into the new tissue. In another polyclad I have obtained very strong experimental evidence which I hope to present at another time in favor of the view that the pressure of the intestinal contents upon the walls of the intestine is a factor of great importance in the formation of intestinal branches or the growth of the intestine into new parts. The differences in the degree of intestinal regeneration in the pieces now under consideration lend strong support to this interpretation. The difference is not merely in proportion to the difference in size, but in the pieces without ganglia the regeneration of the intestine is relatively less complete than in the others. In consequence of the more powerful and frequent muscular contractions of the pieces with ganglia the intestinal contents are pressed against the closed cut ends of the intestinal branches, or, in later stages, into the extensions of these in the new parts with greater force and frequency than in the pieces without ganglia. It is not difficult to observe this difference. The intestinal branches in the new tissue are much more frequently distended by the contents of the intestine in the pieces with ganglia. Thus in regard to these structures as well as those already discussed the differences in the tissues are, I think, expressions of the differences in functional activity, and especially in this case, of muscular activity. The formative conditions in this case, however, are themselves mechanical.

As regards the size of the regenerated genital area posterior to the pharynx there are no marked differences in the two sets. Its size remains almost the same in all cases whether the total amount of regeneration is large or small. This uniformity in size is to be interpreted as indicating that conditions which give rise to these organs are wholly or largely independent of the muscular activity. We know little regarding the conditions which may be concerned in the regeneration of these parts, but they are doubtless connected with the presence of other portions of the ducts in the old part and also with certain obscure physiological conditions on which the presence and periodical activity of the sexual organs is also dependent. The period at which these experiments were performed was not that of greatest sexual activity, and the organs remained



in a more or less rudimentary condition. In a considerable number of other cases, however, animals were used for experiment during the height of the breeding season and the results in these cases are interesting.

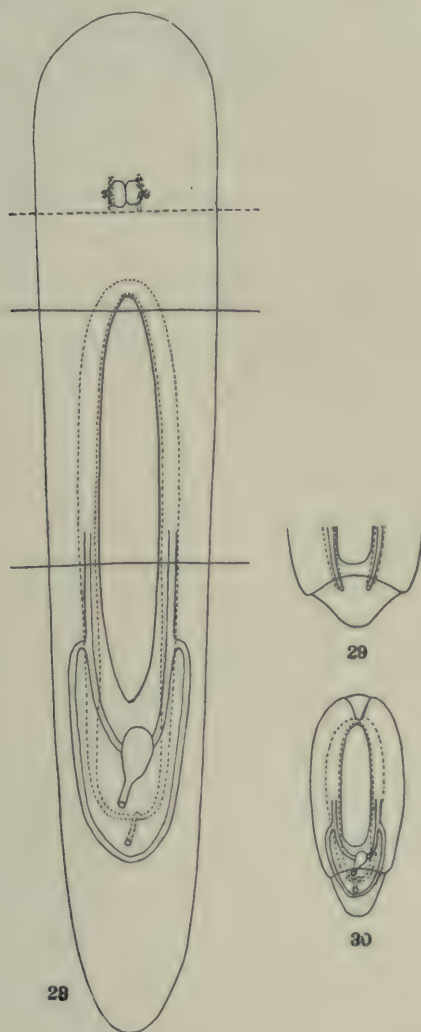


Fig. 28 represents diagrammatically the course of the genital ducts; the female ducts are indicated in broken lines. The



regeneration of many specimens in which these ducts were of large size and filled with the sexual products was observed and it was found that the regeneration of these structures was as complete in the absence of the ganglia as when they were present, though usually somewhat less rapid. The following case will serve as an example: A specimen in the height of sexual activity was cut as in Fig. 28 through the anterior end of the pharynx and again somewhat posterior to the middle of the pharynx, the piece between the two cuts being used for experiment. It will be observed that this piece contained only the anterior portion of the vasa deferentia and a part of the uterus. In Fig. 29 the condition of the piece seventeen days after section is indicated. At this time the ducts were extending into the new tissue but the amount of their regeneration was not clearly visible. Sixty-five days after section the piece presented the appearance of Fig. 30. Complete regeneration of the ducts and the terminal organs had taken place and the ducts were widely distended by sexual products. Similar results were obtained in other cases where the animals used were in a condition of sexual activity. The piece just described was without ganglia, but in other pieces with ganglia the result was the same, though it occurred in a somewhat shorter time.

When nearly all of the body was removed from breeding specimens, *i. e.*, when regeneration occurred from a level only slightly posterior to the ganglia, nothing more than a small genital area, as in Figs. 10 or 11, was ever regenerated even though the ganglia were present. There can be little doubt, I think, that the complete regeneration of the ducts and terminal organs of large size in the case just described was due first to the fact that parts of the ducts remained in the old tissue, and second, and probably chiefly, to the fact that the ducts were in an active functional condition, *i. e.*, filled with sexual products. Here again, as in the case of the intestine, the possible effect of the pressure of the contents of the ducts upon the walls and of other functional conditions upon regeneration must be taken into account. In my opinion these are important factors. In the cases where all parts of the ducts were removed and thus the question as to the effect

of the contents was eliminated the whole apparatus was regenerated in small size and more or less rudimentary form as above stated. But in these experiments the physiological condition of the regenerating specimens as regards sexual activity was the same as in the cases where complete regeneration of the genital apparatus occurred. The only difference is that in the one case all parts of the ducts and all or nearly all of the gonads were removed and there was no possibility of the extreme functional activity of the ducts until new gonads were formed and matured, a process which did not occur in the specimens without food.

In many of my earliest experiments (August) no genital area was observed in the regenerated specimens, though it may have been present in some cases. But later in the autumn, as the breeding period approached its height, the genital area appeared in all cases. This difference indicates that there was some physiological difference between the specimens at different seasons, a difference undoubtedly correlated in some way with the reproductive organs.

In the case shown in Figs. 28-30 it is interesting to note that regeneration of the ducts apparently occurred partly within the old tissue and partly within the new. During the course of regeneration the cut end of the pharynx was gradually retracted from the cut surface, leaving a space behind it in which the copulatory organ appeared. This contraction of the pharynx is probably essentially atrophy from disuse; it occurs only in pieces without ganglia. The region posterior to it is filled in either by cells which have migrated or flowed in from the sides or by proliferated cells or probably by both, and in this the copulatory organ appears. The posterior vasa deferentia extend a considerable distance anteriorly into the old part before they unite with the anterior ducts and the whole system of terminal organs is situated much further anteriorly than originally (compare Figs. 28 and 30). The large size of the regenerated organs is undoubtedly due to the large quantity of the sexual products contained in them and entering them during the course of the experiments. The retraction of the pharynx afforded space for growth, hence the position and extent of the organs.

But the primary object in introducing this case was to show how completely these organs can regenerate in the absence of the ganglia. So far as can be seen they are as perfect as in pieces containing the ganglia.

The conclusions reached from these experiments may be summed up as follows: the influence of the cephalic ganglia upon posterior regeneration is not "formative" for the same organs are regenerated whether the ganglia are present or absent. The amount of regeneration and the size and extent of various organs are, however, greater in the presence of the ganglia. This quantitative difference is probably due to the fact that all functional stimuli and conditions connected with muscular activity and especially those connected with the coördinated muscular activity of locomotion occur with much greater frequency and intensity when the ganglia are present. Probably other functional conditions whose existence is less easily determined are also present in greater degree when the ganglia are present.

c. Posterior Regeneration in the Absence of the Cephalic Ganglia and Parts of the Longitudinal Nerve Cords.

The object of these experiments was to compare the posterior regeneration at a given level in pieces with anterior ends at different levels posterior to the ganglia, in order to determine whether the removal of a considerable part of the nerve cords in addition to the ganglia had any effect on regeneration. In the section on the nervous system and behavior mention was made of the fact that pieces from the posterior region of the body show less motor activity than pieces of the same size from the anterior regions, posterior to the cephalic ganglia. These facts would seem to indicate that the anterior regions of the longitudinal nerve cords differ in some respect—perhaps in complexity—from the posterior regions.

In order to test still further the hypothesis of the relation between posterior regeneration and motor activity pieces with posterior ends at the same level of the body and anterior ends at different levels posterior to the ganglia were used. Certain difficulties were encountered in obtaining definite results from these



experiments. If the level of the posterior ends of these pieces was posterior to the pharynx the amount of regeneration was not very great in any case, and differences were comparatively slight, though apparently in agreement with the hypothesis, *i. e.*, the shorter pieces, those from which larger portions of the nerve cord had been removed, seemed to show somewhat less regeneration than the others, though it was often difficult to be certain that chance individual differences were not concerned. The reasons for the small amount of posterior regeneration from levels near the posterior end of the body have been discussed in the preceding section. It was also found that small pieces from the posterior regions of the body live at most only a few weeks after section, and for some time before death are much contracted and show scarcely any motor activity. The contracted condition of these pieces renders exact measurements impossible.

In order to obtain results at all satisfactory it was necessary to use pieces whose posterior ends were somewhat anterior to the posterior end of the pharynx. With such pieces only the anterior half of the body could be examined in this way, but we are justified in concluding that the same relations between functional activity and regeneration exist in the different regions of the body.

Two series of these experiments afforded definite results. In one of these (Series 69) five pieces were cut as in Fig. 28, the posterior ends being somewhat posterior to the middle of the pharynx and the anterior ends at the anterior end of the pharynx. In the other series (Series 71) five pieces were cut with posterior ends at the same level but with anterior ends just posterior to the cephalic ganglia (see the dotted line in Fig. 28). The two series differ in respect to the region between the cephalic ganglia and pharynx, which is present in one series and absent in the other. Some difference in the motor activity of the two series was noted, the pieces of Series 71 being considerably more active and more successful in locomotion: the differences were, however, not very great. Figs. 31 and 32 represent the two extremes found among the pieces of Series 69 seventeen days after section and Fig. 33 shows the condition of the pieces of Series 71 at the same time, there being little difference among these. In general



the pieces of Series 71 show slightly more regeneration than the others, though the difference is not great.

Thirty-two days after section the pieces of Series 69 had attained the condition of Fig. 34 and those of Series 71 the condition of Fig. 35, the amount being somewhat greater in the latter series. After this time no marked alteration in proportions occurred.

The results obtained from these pieces are not very striking but according to the hypothesis we cannot expect anything more than slight differences in such pieces. Such differences would scarcely attract attention of themselves but when we consider them in connection with the experiments described in the preceding section their significance is apparent. That they are real is well shown by the fact that in certain series which were used for other purposes but which happened to be of such a kind that they could be used in this connection my notes show a difference in the



amount of regeneration similar to that just described, though when the experiments were made the significance of such a difference had not occurred to me and the difference did not attract my attention until examination of my notes and drawings was made several months later.

These results, like those described in previous sections, show a close correlation between the regeneration and the motor activity of the pieces, and I believe that in both cases the conditions connected with the functional activity of the parts are the factors which determine the differences in regeneration.

A comparison of Fig. 35 with Figs. 17 and 18 (Series 79) shows that the amount of regeneration in that piece is greater than in these, although the level from which regeneration occurred is somewhat farther posterior (compare Figs. 12 and 28). If the conclusions reached above are correct less rather than more regeneration should be expected in Series 71 than in Series 79. I believe, however, that a difference in temperature is chiefly

concerned in this difference: in the first place Series 71 was begun October 29 and Series 79 January 9. At the first date the temperature of the water was much higher than on the second and all specimens were much more active. The regenerative power in pieces with ganglia differs markedly with season, *i. e.*, with temperature, as is shown by comparison of Figs. 4 and 5 and 40 and 46, both of which are summer experiments, with Fig. 11, a winter experiment, from nearly the same level. Pieces without ganglia may be expected to show similar though less marked differences.

It is possible that another factor is also concerned: in Series 71 the anterior cut was made as near the ganglia as possible (Fig. 28, dotted line), while in Series 79 it was somewhat further posterior (Fig. 12), the chief object being in this case to remove all parts near the ganglia. In the consideration of anterior regeneration it will appear that the region immediately posterior to the ganglia differs to some extent from other portions of the cords, since its presence determines greater activity and more anterior regeneration; posterior regeneration is probably similarly affected by the presence or absence of this region. It may be, therefore, that the difference between Series 79 and 71 is due in some degree to the absence of this region in the one case and its presence in the other.

#### *d.* Posterior Regeneration in the Head Region.

In all cases of posterior regeneration thus far described the level from which regeneration occurred was one millimeter or more posterior to the ganglia. The piece whose history is given in Figs. 1-5 was cut about a millimeter posterior to the ganglia.

It was found, however, that results differed very little whether the cut surface was one millimeter posterior to the ganglia, or immediately behind them. So long as the ganglia were not greatly injured by the cut, regeneration took place essentially as in Figs. 1-5. But when the cut removed or injured considerable portions of the ganglia the amount of regeneration was only slight and in many cases there was no visible differentiation in the regenerated tissue. The history of one series is given in some detail as an example of these experiments.

*Series 17.* Specimens of large size were selected and the attempt made to separate each into two parts by a transverse cut through the ganglia. It is difficult to make the cut in exactly the desired region and in many cases it passed either anterior or posterior to the ganglia. Ten specimens were finally obtained in which the cut had apparently passed through the ganglia. Only the anterior pieces will be considered at present. Examination showed that some of these pieces contained almost the whole of the ganglia and others only the anterior portions, since the cuts had occurred at various levels in the ganglia. In two pieces (A) the ganglia were very nearly intact, the cut having passed through the posterior part (Fig. 36, the posterior line); in one piece (B) about the anterior half of the ganglia seemed to be present (Fig. 36, the middle line); while in the other seven (C) the cut had passed through the anterior region of the ganglia leaving only small parts, which in some cases protruded from the cut (Fig. 36, the anterior line).

The various pieces showed a marked difference in behavior; the two pieces A behaved like normal animals so far as this was possible in the absence of most of the body, performed coördinated movements and advanced rapidly, though they were not able to adhere closely to the substratum since the posterior parts were absent. The margins of the head were much used in the characteristic manner for pulling the pieces forward.

All of the remaining pieces behaved more or less like pieces from which the ganglia have been removed, but, though they contained parts of the ganglia, they were less active and more helpless than pieces without ganglia but with parts of the longitudinal cords. During the first few days after section little difference could be observed between them. They made no coördinated movements, could not right themselves when reversed, and in most cases simply slid along over the glass by means of their cilia, making irregular contractions. When the ventral surface was brought into contact with the substratum attachment frequently occurred, but the specimens were scarcely able to advance at all. They remained attached for a time and then some muscular contraction usually turned them over and they remained in that position



indefinitely unless righted. Later, presumably after the effect of shock had passed or the injury to the remaining portion of the ganglia had been to some extent repaired, the behavior of the piece B was observed to be somewhat different from that of the pieces C. It was usually found adhering to the substratum and parts of the margins of the body occasionally underwent irregular extensions or contractions; in fact, the movements of this piece were very much like those of *Stylochus*. Occasionally very slow and irregular progression occurred. The pieces C continued to behave as before.

Six days after section:

A. The two pieces are almost identical in appearance (Fig. 37). Regeneration is occurring rapidly and the new tissue possesses the characteristic tapering form with concave lateral margins. The intestinal branches have penetrated the new tissue to a considerable extent. At this time the new tissue was being used for attachment to some extent and was often visibly stretched.

B and C. The one piece B and the six pieces C—one having died—show no marked difference in behavior or regeneration. In some the cut surface is more contracted than in others, but in all the amount of regeneration is very much less than in A. Fig. 38 shows the piece B and Fig. 39 the condition of the pieces C. In the latter a few eye-spots are present but the small pieces of the ganglia cannot be distinguished with certainty; in one case, however, a small rounded mass of cells, probably the remains of the ganglia, was protruding from the dorsal surface just posterior to the cut surface and was apparently fused with the epithelium of the new tissue; this is indicated in Fig. 39 by the shaded area. In none of the pieces did the new tissue show any intestinal branches.

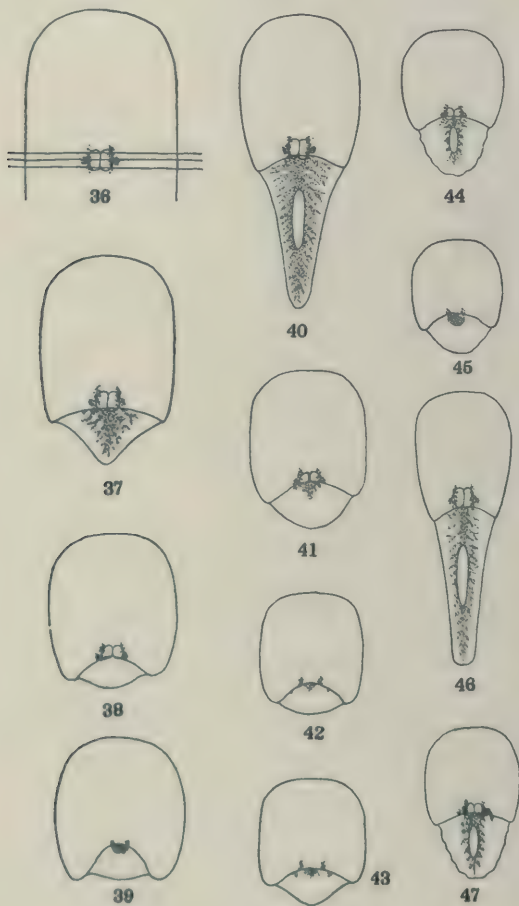
Eighteen days after section:

A. Fig. 40 shows the condition of the two pieces of this group. The new tissue has attained a length greater than the old, the pharynx is well developed and the intestinal branches extend throughout the new part.

B. Fig. 41 shows the condition of the piece B. The new tissue is much less in amount than in A and with few intestinal



branches. No regenerated pharynx was observed. At this time it was noted that the behavior of this piece was slightly different from that of the pieces of the third group. It lay on the dorsal surface less often and sometimes adhered by its margins to the substratum, though progression occurred only occasionally and was very slow, being apparently entirely ciliary.



C. Figs. 42 and 43 represent the two extremes among the four pieces of this group remaining alive. The intestine has penetrated only slightly into the new tissue and no pharynx is

visible in any of the pieces. These pieces are even more helpless than B. Occasionally they adhere slightly to the substratum and rarely slow progression may occur, though movements of progression are less common than in B. When strongly stimulated these pieces usually turn upon the dorsal surface in consequence of their irregular muscular contractions and slide slowly over the bottom of the vessel for a time, but finally come to rest and show only slight muscular contractions.

Twenty-five days after section:

A. The two pieces continue active and normal in behavior and the relative size of the new tissue has increased somewhat though each piece as a whole is undergoing reduction.

B. The behavior of this piece is improving slightly. Though progression occurs only occasionally the piece remains attached to the substratum much of the time. Its movements recall those of *Stylochus*; irregular extensions and contractions of parts of the margin occur frequently, and often in consequence of extension and adhesion of the margin the piece becomes somewhat stretched, not merely longitudinally, but laterally as well. Often a general contraction occurs and all attached regions of the margin are stretched. Fig. 44 shows the form of the piece. The relative size of the new tissue has increased (compare Fig. 41) and a small pharynx is present. The margins of the new tissue are somewhat irregular in form since some parts are more extended than others as the piece adheres to the substratum. Some degree of regeneration of the ganglia seems to have taken place but it is at all events slight.

The small size of the pharynx and the slight extent of the intestinal branches in this piece as compared with A is of interest. The condition of A at this time does not differ greatly from that of a week ago so comparison of the stage Fig. 40 with Fig. 44 will serve to show the differences.

C. Only two pieces are still alive: these are in much the same condition as before, both as regards behavior and regeneration. Fig. 45 shows the piece noted above, in which the remains of the ganglia protrude from the dorsal surface just posterior to the cut.

Thirty-two days after section:

A. Both pieces still active and normal in behavior; both are like Fig. 46 in form. The length of the new tissue is much greater than that of the old and the concave outline of the lateral margins is still evident.

B. As regards behavior this piece is in the same condition as a week ago. Its form is shown in Fig. 47. The relative amount of new tissue has increased somewhat during the week and the pharynx is larger. The great difference in form and structure between the new part of this piece and that of the pieces A both at the present and earlier stages should be noted (compare Fig. 47 with Figs. 37, 40 and 46). The form is widely different from the form of the new part in A at the stage when about the same amount of new tissue is present (Fig. 37). I can conceive of no good reason for this difference except the difference in mechanical conditions to which the corresponding parts have been subjected in A and B in consequence of the difference of motor activity. At the present stage (compare Figs. 46 and 47) the difference in size of pharynx and extent of intestinal branches in A and B is striking. These differences can scarcely be due to anything but differences in functional activity of the parts in the two pieces.

C. All are dead.

Of the three remaining pieces B died within the next two days, then one of the two pieces A, and finally the other.

The history of the series shows very clearly the correlation between activity, capacity for regeneration, and length of life on the one hand and the amount of ganglionic tissue present on the other. Other series of the same sort afforded similar results, though in no case were the differences so well shown in a single series. The questions as to the amount of regeneration, form of the regenerated parts, size of pharynx, and extent of intestine which were discussed in the consideration of posterior regeneration in the absence of the ganglia receive the same answers from this series as from those already considered. The formation of new tissue, but without differentiation in the characteristic manner, in the pieces of the group C is, I think, of especial interest as indicating that the conditions of the two processes are different,



thus confirming the suggestion already made regarding the earlier stage of regeneration (pp. 487 and 488).

In all cases pieces from the region anterior to the cephalic ganglia died within two or three days after section. In some of these a small amount of unpigmented "new" tissue appeared upon the cut surface but the early death of the pieces prevented further regeneration.

*e. Résumé of Experiments on Posterior Regeneration.*

The experiments described show that the presence of the cephalic ganglia is not necessary for regeneration in the posterior direction at any level posterior to the ganglia. When they are present the size of the regenerated part is larger but regeneration is qualitatively complete in their absence. From these facts we are justified in concluding that no "formative stimuli" for the parts under consideration proceed from the cephalic ganglia. The relation between the ganglia and the amount of regeneration, on the other hand, shows that the conditions upon which regeneration depends are quantitatively affected by the presence of the ganglia. As I have endeavored to show, the facts indicate that at least certain of these conditions are those resulting from the characteristic motor activity of the animal; probably other functional conditions besides those connected with movement are also concerned, though their existence is less readily demonstrated.

The difference in motor activity of the posterior parts between the pieces with and those without cephalic ganglia is essentially one of degree; in the absence of the ganglia all conditions and stimuli connected with motor activity are less intense and occur in less orderly sequence, though very probably none are wholly absent. If these are "formative factors" it follows that the regenerative processes must occur in less degree in the absence of the ganglia. According to this view the relation between the ganglia and regeneration is indirect, not direct; the ganglia are not the "centers" for special formative stimuli, but are merely more or less complex portions of the nervous system connected on the one hand with certain sense organs and on the other with other parts of the nervous system and so with the various regions of the



body. In consequence of these connections complex series of movements and other functional activities which we designate as coördinated are possible. The more complete the system the more perfect the coördination and vice versa. When parts are removed the functional activities of the remaining parts determine what shall regenerate in place of the absent parts; these functional activities being dependent upon the nervous system an apparent correlation between parts of the nervous system and the character of regeneration exists. I see no necessity, however, for assuming the existence of special formative stimuli in this case; further discussion may be postponed to another time.

As regards the question of "centers" in the nervous system my views are in essential agreement with those of Loeb ('99). The experiments do not indicate the existence of special "centers" in the old sense in the nervous system. I have pointed out, however, that the complexity of behavior seems to be determined in some degree by the amount of the nervous tissue present. In this respect the cephalic ganglia and the longitudinal cords differ greatly in importance. In general the greater the portion of the longitudinal cords remaining intact the more complex and coördinated is the character of the activity. But even when the cords are intact throughout the activity is far below the normal activity in complexity and degree of coördination. With the addition of a part of the tissue of the cephalic ganglia to the longitudinal cords there is a marked increase in the complexity and coördination of movement, and when half or more of the ganglionic tissue is present the behavior approaches that of the normal animal. On the other hand after complete removal of the cords and half or less of the ganglionic tissue the behavior is almost normal, but when more than half of the ganglionic tissue is absent the complexity of behavior falls to a low level. Moreover, as far as I can determine, it makes no difference whether the part removed be anterior, posterior or lateral; it is primarily the amount not the particular region that determines the result. These facts are of importance as indicating that the nervous system is fundamentally a system of connections permitting the transference and doubtless also the accumulation and transformation of stimuli,

and that particular functional capacities are primarily dependent not upon a given part but upon the relations between that part and others.

In another paper further data will be given in the consideration of the relation between the central nervous system and anterior and lateral regeneration.

Before concluding, the study by Schultz ('02) of regeneration in *Leptoplana atomata* requires brief comment. Schultz being chiefly concerned with the morphological and histological features of the process made no comparative study of regeneration from different regions. He states that posterior regeneration is complete, but apparently has not examined pieces from the region anterior to the ganglia; in all his specimens the cuts were made posterior to the ganglia. Schultz is inclined to deny that the ganglia are connected in any way with regeneration, but his experiments along this line concern chiefly anterior regeneration and will be considered at another time. Experiments on posterior regeneration from levels posterior to the ganglia and in their absence were apparently not made by him. So far as his experiments are adapted to the problem which I have considered it appears that *L. atomata* agrees essentially in all respects with *L. tremellaris*. But since his work has a purpose widely different from that of my own it affords no further data for present consideration.

#### D. SUMMARY.

1. Pieces of *Leptoplana* deprived of the cephalic ganglia react to stimuli less strongly than normal animals and to some not perceptibly. A considerable degree of motor activity may be present, and progression is possible but all movements are indefinite and to a large extent uncoordinated. The behavior of pieces without ganglia differs somewhat according to size (amount of nervous tissue present) and the level of the section (qualitative differences in different regions of the cords). Pieces from the region anterior to the ganglia show scarcely any motor activity except ciliary movement and die within a few days.

2. Removal of about half of the tissue of the cephalic ganglia does not appreciably diminish motor activity or coördination, but

when larger portions are removed the behavior resembles that of pieces without ganglia. The result is the same whether the part removed be anterior, posterior, or lateral.

3. In general the experiments indicate that the amount of nervous tissue, *i. e.*, the completeness of the system of connections between parts, rather than the presence of particular regions is the important factor, in determining motor activity. The results support the view that particular centers or regions in which certain stimuli originate do not exist.

4. In considering relations between the nervous system and morphogenesis it is important to determine whether the relations are direct or indirect, *i. e.*, whether certain nerve stimuli are themselves formative stimuli or whether the conditions connected with the functioning of the part in a characteristic manner are the true formative stimuli, the nervous stimuli being merely the determining factors of the function. The experiments on *Leptoplana* indicate that the relation between the nervous system and regeneration is, at least in large part, indirect.

5. Posterior regeneration is qualitatively complete at all levels posterior to the cephalic ganglia, though in the absence of food the regenerated part never attains the size of the part removed, *i. e.*, the further posterior the level from which regeneration occurs the less is the amount of regeneration. The only satisfactory explanation of this difference is the difference in functional stimuli and conditions to which the regenerating part is subjected at different levels; the further posterior the level from which regeneration occurs the less the new part is used.

6. The difference in the amount of regeneration at different levels is to a large extent independent of the size of the piece. Small pieces from the anterior region of the body may equal or exceed in amount of posterior regeneration pieces several times as large from the posterior regions.

7. Comparison of posterior regeneration from a given level in pieces with and those without ganglia shows the following differences; the amount of regeneration is less in the pieces without ganglia, but regeneration is qualitatively complete in both; the form of the regenerated part is less elongated and usually less



tapering, the pharynx is smaller and the intestinal branches in the new tissue are shorter and less numerous in pieces without ganglia; the differences between pieces with and those without ganglia are much greater during later stages of regeneration than during the earlier; and they are also greatest when regeneration occurs from a level not far posterior to the ganglia, and decrease as the level of the cut surface approaches the posterior end.

8. The effect upon regeneration of the removal of parts of the ganglia depends upon the amount of tissue removed. Removal of about half the ganglionic tissue is followed by complete regeneration, but if more than half be removed regeneration is slight. The result is the same whether the part removed be anterior, posterior, or lateral. The relations between the ganglia and regeneration are thus similar to those between the ganglia and motor activity (see 3).

9. The experiments indicate that the cephalic ganglia do not exercise any "formative" influence upon posterior regeneration. The differences observed between pieces containing the ganglia and those without them are readily explained if the conditions connected with functional activity of the parts, and in these cases especially motor activity, are regarded as formative factors. Thus the relation between the nervous system and posterior regeneration is indirect, not direct. The presence of some part of the central nervous system is, however, necessary for the continued existence of a piece and so indirectly for regeneration.

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## STUDIES ON REGULATION.

### VI. THE RELATION BETWEEN THE CENTRAL NERVOUS SYSTEM AND REGULATION IN LEPTOPLANA: ANTERIOR AND LATERAL REGENERATION.

BY

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WITH 64 FIGURES.

#### A. ANTERIOR REGENERATION.

Anterior regeneration in *Leptoplana* differs widely from posterior regeneration (Child, '04b) as regards its apparent relation to the central nervous system. In the absence of the cephalic ganglia it is very incomplete both qualitatively and quantitatively: regeneration of the "head" never occurs when the ganglia are absent.

The work of Schultz ('02) upon polyclads has afforded similar results, but the interpretation given by Schultz differs widely from that offered below. Schultz denies the existence of a relation of any kind between the central nervous system and anterior regeneration and believes that the almost complete absence of anterior regeneration from levels posterior to the ganglia is due to the fact that the margins of the cut surface unite and so present a mechanical obstacle to regeneration. As will appear below this is certainly not the case in *Leptoplana tremellaris* and probably not in *L. atomata*, the species employed by Schultz for his experiments. Schultz's experiments were apparently confined wholly to the region posterior to the ganglia, where a head never regenerates: if he had extended his examination to the region of the ganglia and that anterior to them there is little doubt that he would have reached very different conclusions.

The relation between the cephalic ganglia and motor activity

was described in a preceding paper (Child, '04a). It was found that removal of more than half of the ganglionic tissue is followed by a marked reduction in the power of coördination and motor activity in general, though pieces without ganglia are capable of slow progression and are able to right themselves when turned over. A relation between the cephalic ganglia and the amount of posterior regeneration was found to exist, but all the organs removed are regenerated in the absence of the ganglia though in smaller size or in some cases of less complexity than when the ganglia are present. The various conditions to which the parts are subjected in consequence of motor activity and other functional activities are undoubtedly formative factors, as has been demonstrated by experiment for certain cases (Child, '02, '03, '04a), and since the motor activity is dependent in large degree upon the presence of the cephalic ganglia the relation between the ganglia and the amount of posterior regeneration is to be regarded as indirect rather than direct, *i. e.*, the ganglia themselves do not give rise to special formative stimuli but determine and regulate the functional conditions and so exert an influence upon regeneration.

It now remains to consider whether the conditions of anterior regeneration are similar to those of posterior regeneration or whether additional factors are concerned. The differences in anterior regeneration from different levels in relation to motor activity will serve as a basis for this consideration.

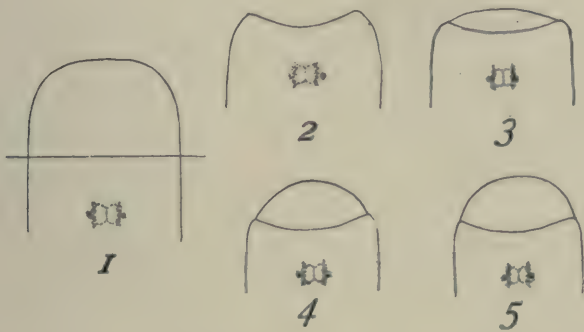
The figures are drawn in the same manner and on the same scale as in preceding papers.

#### 1. *Anterior Regeneration from Levels Anterior to the Cephalic Ganglia.*

Removal of any part of the head anterior to the cephalic ganglia is followed in all cases by rapid regeneration. It makes little difference whether a small part only or the whole region anterior to the ganglia is removed. The larger the part removed the longer the time required for complete regeneration, although the rapidity of regeneration increases with the size of the part

removed, so that regeneration of a large part of the head requires only a slightly longer time than regeneration of a small part.

Figs. 2-5 illustrate the course of anterior regeneration after removal of the anterior portion of the head by a cut at the level of the transverse line in Fig. 1. In this series (Series 16, August 16, '02) ten specimens were subjected to the operation and the results obtained were essentially similar in all. After section the cut surface contracts and becomes concave (Fig. 2). Six days after section (Fig. 3) a mass of new tissue with convex margin is visible on the cut surface. Fourteen days after section (Fig. 4) this mass has increased in size and is approaching in form the normal head. Eighteen days after section (Fig. 5)



regeneration is practically completed. Some slight increase in the new tissue may occur after this time but it is not sufficient to alter the measurements to any marked extent. The distribution of the intestinal branches in the new tissue is only slightly less complete than in the normal animal (not shown in the figures). Comparison of the figures shows that the old cut surface has become less concave during regeneration until in Fig. 5 it is nearly a plane surface again. Numerous other specimens subjected to similar operations afforded similar results.

The behavior of these pieces during regeneration is, as might be expected, similar in most respects to that of normal animals. There is, however, a characteristic difference in the use of the mutilated head. The continual searching movements of the



margins of the head during creeping are familiar to all who have observed turbellarian movements. In the absence of the anterior part of the head, which is the chief organ for these movements, the other parts are apparently used to a greater extent than usual and the new tissue becomes functional early in the course of regeneration, being also extremely active. Discussion of the question as to the relation between the motor activity and regeneration is postponed until the results of section at other levels have been considered.

## 2. *Anterior Regeneration after Section Through the Ganglia.*

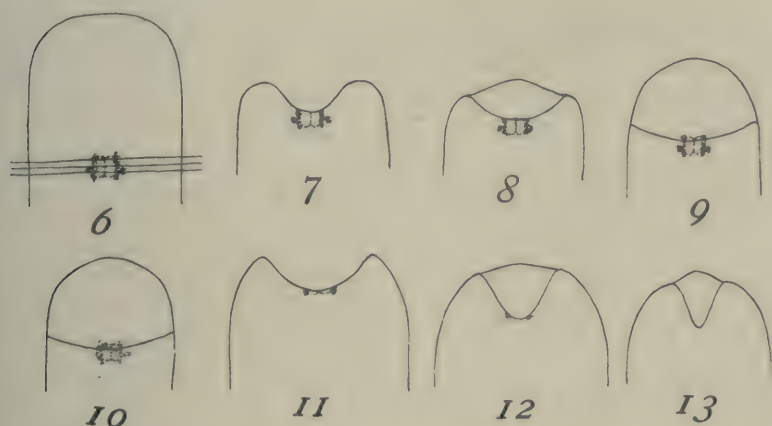
The results of section through the cephalic ganglia differ according to the amount of ganglionic substance which remains intact. In cases where the plane of section passes through the anterior half of the ganglia the course of regeneration is essentially similar to that after section anterior to the ganglia, but if the greater part of the ganglionic substance is removed the regeneration is less rapid and is usually very incomplete.

The history of a single series will serve to illustrate these points. In Series 17 (August 16, '02) ten specimens were cut transversely as nearly as possible through the middle of the ganglia. There was some variation in the position of the cut in the different specimens: The three transverse lines in Fig. 6 will show approximately the levels of the cuts: in seven cases the cut was near the level of the anterior line, in one near the level of the middle line, and in two, one of which was lost, it was near the level of the posterior line. In the first eight pieces in which the cut passed through the middle or anterior part of the ganglia anterior regeneration was complete; in the ninth in which only the posterior portion of the ganglia remained it was very incomplete.

The posterior regeneration of the anterior pieces of this series was described in the preceding paper (Child, '04b). It will be remembered that seven of the anterior pieces (Group C) in which the cut was anterior to the middle of the ganglia showed only slight posterior regeneration, one piece (B) in which the cut passed somewhere near the middle region of the ganglia regenerated somewhat more completely, and two pieces (Group A) in

which the cut was near the posterior region of the ganglia showed complete regeneration.

The eight posterior pieces of this series corresponding to Groups B and C of the anterior pieces all showed complete regeneration; the ninth posterior piece corresponding to one of the two anterior pieces comprising Group A regenerated only very slightly, the posterior piece corresponding to the other piece of Group A was lost. This comparison of the anterior and posterior pieces shows very clearly that regeneration, whether posterior or anterior, is most complete in these pieces which contain the largest amount of ganglionic tissue. In the piece B described in the preceding



paper posterior regeneration was qualitatively complete though much less in amount than in Group A; the posterior piece corresponding to B showed complete anterior regeneration. Thus in this case the ganglionic tissue was divided so evenly that both pieces retained some considerable portion of it intact. If it were not for the crushing and displacing effect of the cut upon the soft tissues it would doubtless be possible to cut through the ganglia transversely in such manner that both posterior and anterior pieces would regenerate completely. This case is, however, the nearest approach to success which I have obtained in numerous experiments of this kind.

The history of the first eight pieces is illustrated by Figs. 7-10.

Fig. 7 shows the contracted condition of the cut surface after section, the contraction being much more marked than in Fig. 2. Fig. 8 shows the condition six days after section; new tissue is growing out and the concavity of the cut surface is already decreasing. Fig. 9 represents a stage fourteen days after section; the new tissue is acquiring the characteristic rounded outline of the head and the cephalic ganglia are regenerating. In Fig. 10, eighteen days after section, regeneration is essentially complete. The new tissue has attained about the same form as the part removed, though it is not actually as large, the whole body having decreased in size in the absence of food. The concavity of the cut surface is nearly obliterated and the anterior intestinal branches—not shown in the figures—are distributed in the characteristic manner throughout the new tissue. Eyes have appeared in the new tissue and the cephalic ganglia are apparently fully regenerated. The piece in which the cut passed near the middle of the ganglia differs from the other seven only as regards the ganglia, which are somewhat smaller than in the other pieces.

In the ninth piece, however, in which only a small part of the ganglionic tissue remained, the results were very different. Figs. 11-13 represent the various stages. After the contraction following section (Fig. 11) it was not possible to distinguish with certainty the remaining portions of the cephalic ganglia though the piece possessed some eyes. In Fig. 12 the condition of this piece six days after section is shown. It will be observed that the concavity of the cut surface has increased instead of diminishing as in the other cases. New tissue has filled the angle between the two sides of the cut surface but does not extend beyond it. The eyes have apparently degenerated, none being found at this stage. No evidence of regeneration of the cephalic ganglia is visible. From this condition the piece gradually changed to the condition represented in Fig. 13 eighteen days after section. Here the contraction of the cut surface has proceeded still farther and the small amount of new tissue has apparently been pushed out from between the two approaching surfaces. The new tissue does not form a head, no ganglia or



eyes are present, and the intestinal branches scarcely enter it. The margin of the new part is similar to the margins of the adjoining old portions but no special differentiation of any kind can be observed. It is probable that the small portions of the cephalic ganglia remaining after section have undergone degeneration. No further advance in regeneration occurred even after months.

No less striking than the difference in regenerative power between the eight pieces containing half or more of the ganglionic substance and the one piece containing only a small part is the difference in their behavior. The eight pieces behaved throughout essentially like normal animals. I thought I could distinguish a slight difference between them and uninjured specimens as regards rapidity and precision in locomotion, which might be expected from the absence of the chief tactile organ, the margin of the head, but the remaining parts of the head and the new tissue as soon as it became functional were even more active than these parts in uninjured animals; in the absence of the usual tactile and other stimuli from the anterior regions of the head the parts present were used all the more. The ninth piece, however, resembled in behavior a piece without cephalic ganglia. It was able to advance only slowly, did not adhere closely to the substratum, the muscular movements were irregular and ineffective, and when turned upon its back the piece regained the normal position only after some time and many ineffective muscular contractions of various parts. The continual muscular play of the lateral margins of the head was almost wholly absent in this piece. In all probability the piece was practically without ganglia in consequence of the degeneration of the small portions of the ganglia remaining after section.

There can be little doubt that in this piece the region of the cut surface is subjected to conditions differing widely from those present in the other eight. In the first place all conditions connected with the normal rapid progression are absent; the contacts with the substratum are much less close; owing to the lack of coördination and reactive power the conditions resulting from muscular movements of the head region are to a large extent absent; peristaltic and other muscular contractions of the whole body



or of parts are much less powerful, and consequently the pressure exerted upon the anterior region by such contractions through the intestinal contents or other fluids in the body are greatly reduced. In the other eight pieces the head region functions all the more actively because parts are missing and the stimuli received from them are absent or received in a new manner. In this piece, however, the movements of the head region are very slight. In short, all or nearly all the functional conditions characteristic of the head region are absent or greatly reduced. Here again as in connection with posterior regeneration (Child, '04b) we find a close parallelism between functional activity and power of regeneration. The significance of the facts will become still more evident in the light of further data given below.

Description of other series would only multiply details without adding anything essential. All specimens cut through the middle or anterior half of the ganglia behave much like normal animals and regenerate rapidly and completely while those in which only a small part of the ganglia remains behave much like specimens without ganglia, never regenerate a head, and apparently lose the small portion of ganglionic tissue by degeneration. The amount of new tissue regenerated is sometimes more and sometimes less, but, as will appear below, various conditions may determine such differences.

In the preceding section (p. 514) the statement was made that the greater the part of the head removed anterior to the ganglia the greater the rapidity of regeneration. This fact is well illustrated by a comparison of the series described in that section (Series 16) with Series 17 described above. Both of these series were begun on the same day and both were examined at the same intervals so the results are strictly comparable. Figs. 2, 3, 4 and 5 represent stages of Series 16 corresponding, respectively, to the stages of the eight pieces of Series 17, shown in Figs. 7, 8, 9 and 10. Fourteen days after section the pieces of Series 17 (Fig. 9) have regenerated about twice as much tissue as those of Series 16 (Fig. 4). Eighteen days after section regeneration in both is about complete, though the pieces of Series 17 (Fig. 10) have had about twice as much material to replace as those of Series 16

(Fig. 5). I am inclined to believe that functional conditions may account in large part for this difference. The larger the part which the new tissue represents the greater and more varied is its activity and if the various conditions connected with this activity affect growth in any way a more or less exact proportionality between the rapidity of regeneration and the size of the part removed may be expected.

3. *Anterior Regeneration from Levels Immediately Posterior to the Cephalic Ganglia.*

Anterior regeneration from levels only a short distance posterior to the cephalic ganglia differs in certain respects from that occurring from levels farther removed from the ganglia. Although nothing like a head is ever regenerated the amount of regeneration is usually somewhat greater than at other levels posterior to the ganglia and the new tissue possesses a somewhat different form. Individual differences which occur are doubtless due in part to slight differences in level of the plane of section, though, as will appear below, some cases indicate that internal factors differ in different individuals. The history of a series will serve to illustrate these points.

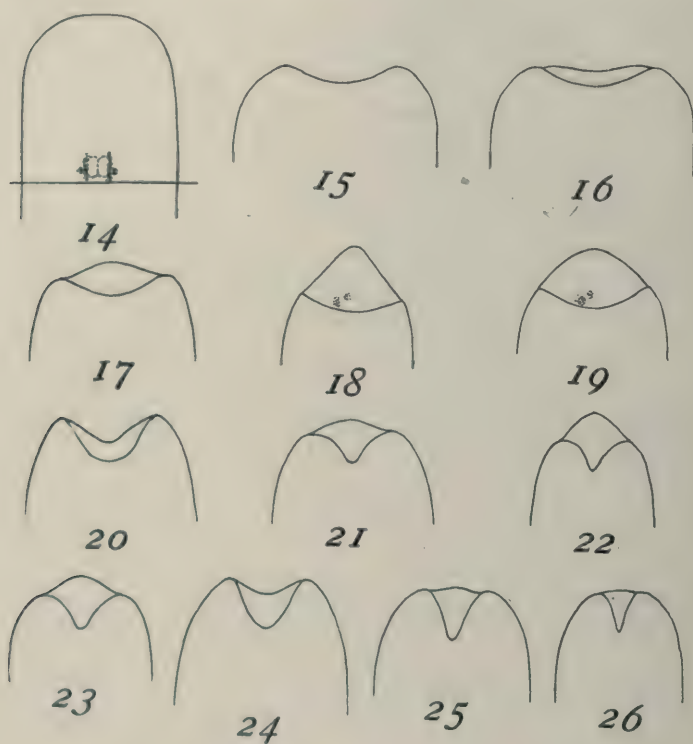
*Series 71.* The head and ganglia were removed from a number of large specimens by a cut just posterior to the ganglia as in Fig. 14. In all cases this cut was made as near as possible to the eyes, but without including any part of them in the posterior piece. All pieces were examined after section and those in which eyes were present in the posterior piece or in which the plane of section lay too far posteriorly were discarded. Five posterior pieces were finally obtained for the experiment.

A few hours after section all pieces appeared much like Fig. 15; contraction of the cut surface had occurred and as is usual in pieces without ganglia considerable longitudinal contraction had occurred so that the width of the body was greater than before section.

The pieces were examined every few days and the following figures show the condition of the various pieces six, seventeen and thirty-two days after section. Three types were recognizable as

regards the amount and form of the new tissue; these are designated as A, B and C.

A. (Figs. 16-19.) One piece only regenerated in this manner. It will be observed from the figures that in this case the contraction of the cut surface was greater than in pieces containing ganglia (see Figs. 2-5), and that the new tissue grew out from the wound



as a rounded mass (Fig. 17), which later became somewhat pointed (Fig. 18). Moreover, two groups of pigment spots, undoubtedly eye-spots, appeared in the new tissue just anterior to the cut surface. Regeneration never proceeded beyond the condition represented in Fig. 18. No trace of regenerated cephalic ganglia could be observed at any time. Fig. 19 represents the contracted condition of the same stage; the form shown in Fig. 18 appeared



when the piece was creeping; the other, Fig. 19, when the piece was at rest.

B. (Figs. 20-23.) Three pieces regenerated in this manner. Here the contraction of the cut surface was somewhat greater than in A and continued to increase throughout the experiment. The new tissue grew out from the wound at first in rounded form (Fig. 21), but later acquired the tapering form shown in Fig. 22. No traces of ganglia or eyes appeared at any time. The differences between the three pieces were slight. Figs. 22 and 23 represent, respectively, the extended form and the form during quiescence of these pieces. The new tissue was more pointed during the former condition.

C. (Figs. 24-26.) One piece regenerated in this manner. The course of regeneration in this case was much like that from more posterior levels; the contraction of the cut surface was greater than in the other cases and continued to increase. The new tissue filled the space between the sides of the wound, but never extended beyond the rounded margins of the old tissue. In this piece the differences in form during movement and rest were very slight.

All of the pieces showed a somewhat greater degree of motor activity and power of coördination than pieces from which the anterior third or more of the body had been removed. After stimulation progression continued for a considerable time and the pieces changed their positions in the dishes from time to time without perceptible external stimulation. Differences in motor activity among the different pieces were not great but it seemed to me that the pieces A and B were somewhat more active than C, though in all cases of this kind differences are not strongly marked. In A and to a less extent in B the new tissue occasionally showed movements resembling the searching movements of the head in normal animals and some slight irregular play of the margins of this region was observed. In C nothing of this kind occurred. The changes in form of the anterior ends in A (Figs. 18 and 19) and B (Figs. 22 and 23) are of interest; when the pieces extended and advanced the new tissue became somewhat more slender and pointed as if it were pushed forward by internal



pressure, while during rest it assumed a more rounded form. This change in form is doubtless due primarily to muscular contractions; the decrease in transverse diameter in consequence of muscular contraction during progression must produce pressure in the direction of the longitudinal axis at the anterior end—and at the posterior end also if this is a cut surface: this pressure must bring about elongation of the new parts; hence the change of form in these regions. There can be little doubt that this pressure constitutes a factor in the outgrowth of new tissue from a cut surface and the form which it acquires. If this is the case the greater outgrowth in A and B may be due in part to the fact that such pressure has been more frequent or perhaps greater in amount than in C. The different degrees of contraction of the cut surface in the different pieces are also very probably due to this or other similar factors connected with motor activity.

The appearance of eyes in A is probably due to the outgrowth of nerves from the cut end of the cords and their union with the epithelium.

These five pieces, which were cut as nearly as possible at the same level, afford a good illustration of the difficulty of complete control of experiments of this kind. It is very probable that the differences are due to slight differences in level of the cut or in the extent of injury to the tissues posterior to the cut surface, but it is impossible to determine with certainty whether this is the case. The points of chief importance are, however, the occurrence of a considerable amount of anterior regeneration (in A and B) in the absence of the ganglia and the somewhat greater degree of motor activity in all of these pieces, and especially in A and B, as compared with regions further posterior. In this connection it is also of interest to note that regeneration was more rapid in A than in B and in B than in C as may be seen by comparing the three sets of figures. Thirty-two days after section A (Figs. 18 and 19) had regenerated more than twice as much new tissue as B (Figs. 22 and 23) and several times as much as C (Fig. 26). But even in A regeneration is much less rapid and the total amount is less than in pieces containing the ganglia. Comparison of Figs. 2-5 and 8-10 with Figs. 15-18 will illustrate this fact. In

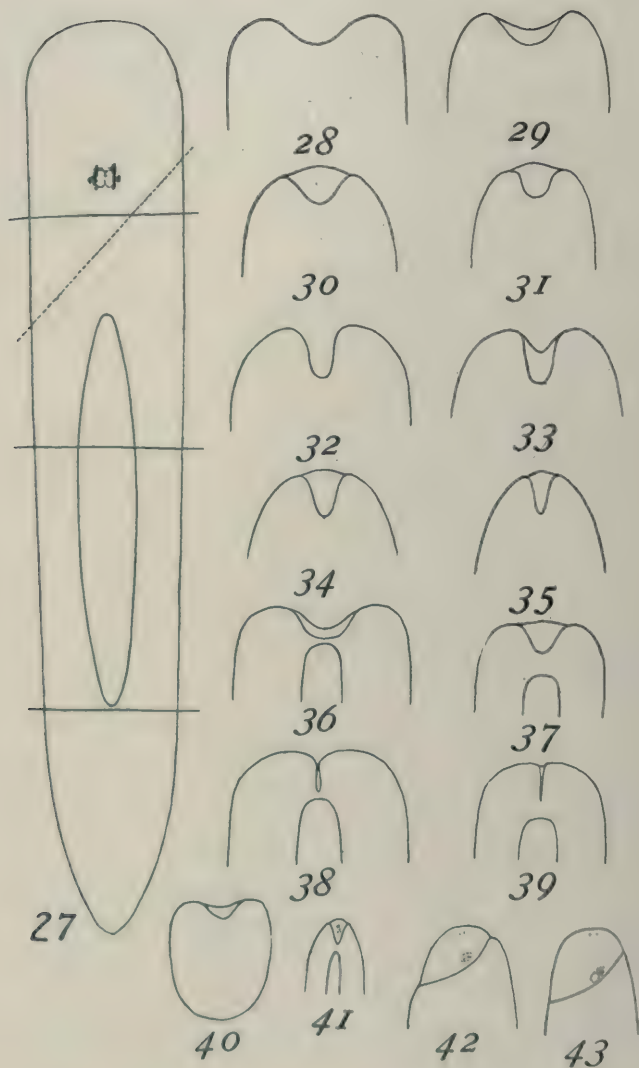
these pieces differences of this kind cannot be correlated with differences in the size of the part removed, since this was approximately the same in all cases. They are rather to be compared with the cases described in the preceding paper (Child '04b) in which the rapidity and amount of posterior regeneration from a given level was much greater in the presence of the ganglia than in their absence, and I believe that they are to be explained in the same manner, viz: as due chiefly to differences in motor activity.

Numerous similar experiments afforded similar results. In no case did a characteristic head regenerate in the absence of the ganglia, but regeneration from a level immediately posterior to the ganglia was in almost every case more rapid and greater in amount than from more posterior levels. Moreover, whenever differences in motor activity could be distinguished the most active pieces showed the greatest amount of regeneration.

#### 4. *Anterior Regeneration from Other Levels Posterior to the Cephalic Ganglia.*

When both the ganglia and the region immediately posterior to them are removed, anterior regeneration is usually even less in amount than in the cases above described; occasional exceptions to this rule occur, however, some of which will be described below. But in general the amount of anterior regeneration from these levels is inversely proportional to the size of the part removed, or in other words, the farther posterior the level from which it occurs, the less the regeneration. This relation is exactly the reverse of that observed in connection with posterior regeneration (Child, '04b); there the amount of regeneration is directly proportional to the size of the part removed.

My experiments along these lines include nearly two hundred specimens but with only one marked exception as regards the amount of new tissue and three cases in which a few eye-spots appeared. Experiments in three different regions are selected for description. The levels are indicated by the three unbroken transverse lines in Fig. 27. The results obtained from section at the most anterior of these three levels are indicated in Figs. 28-35. Figs. 28-31 represent the history of a piece in which the cut surface



remained widely open and Figs. 32-35 that of a piece with margins of the cut approximated. These two pieces represent the extreme forms obtained after section at this level. The stages represented are as follows: Figs. 28 and 32, a few hours after section; Figs. 29 and 33, six days after section; Figs. 30 and 34,

seventeen days after section; Figs. 31 and 35, thirty-two days after section. These two pieces belong to series which were begun on the same day and examined at the same intervals as Series 71, described in the preceding section. Figs. 28 and 32 may then be compared with Fig. 15; Figs. 29 and 33 with Figs. 16, 20 and 24; Figs. 30 and 34 with Figs. 17, 21 and 25; and Figs. 31 and 35 with Figs. 18, 22 and 26. This comparison shows that the regeneration in these two pieces is less than in Series 71, a result in accordance with the general statement made above since the level from which regeneration occurs is somewhat further posterior in these pieces than in Series 71.

Other series from this level do not differ essentially from these cases.

The anterior regeneration after section near the middle of the pharyngeal region is indicated in Figs. 36-39. Figs. 36 and 37 represent one extreme in which the wound remains open, and Figs. 38 and 39 the other in which the contraction is so great that the margins of the cut surface come into contact. Figs. 36 and 38 represent stages six days after section and Figs. 37 and 39 stages a month after section; Figs. 37 and 39 may be compared with Figs. 31 and 35, and Figs. 18, 22 and 26. The amount of regeneration is small when the edges come into contact as in Fig. 38. The two surfaces simply unite and no further growth occurs. This contact of the cut edges is very common in the pharyngeal region and probably results from the gradual retraction of the cut end of the pharynx. In consequence of this the lateral parts of the wound are brought into contact, since in the absence of the pharynx the cavity in the median region collapses. The condition with the edges of the cut surface in contact or nearly so is more common in the pharyngeal region than that represented in Figs. 36 and 37.

Anterior regeneration from levels posterior to the pharynx is very slight. In pieces from this part of the body the cut surface does not usually close as it does in pieces from the pharyngeal region since here there is no large median cavity. Fig. 40 shows the condition thirteen days after section of a piece which represents the whole of the post-pharyngeal region (Fig. 27) and indi-



cates the maximum amount of regeneration obtained in cases of this kind. In most cases such pieces die within ten days after section and none were kept alive more than twenty-eight days. Shorter pieces cut further posteriorly do not live as long and show even less regeneration than these.

These three examples are sufficient to indicate the decrease in the power of anterior regeneration with approach toward the posterior end of the body. The results obtained in other series were in general similar.

As regards motor activity the differences at different levels are parallel to the differences in regenerative power. The further posterior the level of section the less the motor activity. Pieces from widely different levels like those considered above (Fig. 27) show marked differences in motor activity. The pieces of the first series, which had lost only the ganglia and a short portion of the cords, were almost as active as Series 71 considered in the preceding section, which had lost only the ganglia; progression occurred, though of course slowly, and the pieces were able to adhere to the substratum in some degree and to right themselves when turned over. The special activity of the anterior end, present in some degree in Series 71, was not observed in these pieces. The pieces with anterior ends near the middle of the pharyngeal region were distinctly less active. When progression occurred it was less rapid than in the first set, direct comparison between pieces of the two kinds being frequently made; the pieces righted themselves less readily and sometimes did not succeed at all; adhesion to the substratum was very slight; and finally all movements were less frequent and powerful. But the pieces from the region posterior to the pharynx showed even less motor activity. Very slow progression sometimes occurred in consequence of ciliary movements, but in such cases the piece simply slid along without holding to the substratum and frequently on the dorsal surface. The pieces were usually incapable of righting themselves. Reactions to stimuli were slight and the pieces showed few traces of movement of any kind when left undisturbed. In the normal animal this region of the body is the chief organ of attachment during locomotion, but separated from other parts it

is incapable of more than a very slight degree of attachment. In the absence of the mechanical tension to which the tissues are normally subjected, the apparent reduction in size of these pieces is considerable (compare the posterior part of Fig. 27 with Fig. 40. See also Child, '02). This apparent reduction is due in part to altered physical conditions, for the decrease in the longitudinal and transverse diameters is accompanied by a relative increase in the dorso-ventral diameter. In general the same parallelism between motor activity and regenerative power is found in these pieces from different regions as in those already described.

Brief mention must be made of a few special cases of anterior regeneration which differ in certain respects from the average. Groups of eye-spots appear occasionally in the new tissue of posterior pieces not only when the plane of section is immediately posterior to the ganglia as in piece A, Series 71 (p. 522) but even at levels as far back as the anterior end of the pharynx. Except where the plane of section is very near the ganglia the eye-spots appear only after two or three months. In Fig. 41 a case of this sort is shown; here the cut was made at the level of the anterior end of the pharynx and the eyes were first observed two and one-half months after section. Other cases do not differ essentially; sometimes only one or two eye-spots appear and sometimes two or three groups of them are visible. It is probable that outgrowth of nerves from the cut ends of the nerve cords is responsible for the formation of these structures. If this is the case it is an interesting fact that the eye-spots are developed in such cases in connection with a part of the nervous system different from that with which these organs are usually connected. This is another bit of evidence in favor of the view that the differences between different regions of the central nervous system are differences of degree rather than of kind. Regions of the body posterior to the anterior end of the pharynx have never been seen to produce eye-spots; on the other hand they are most common in pieces like A, Series 71, from which only the ganglia have been removed. The frequency of regeneration of these organs decreases posteriorly like the power of regeneration and of motor activity. Probably these organs are the result of a direct influence of the nervous

system. Another exceptional case was that of a piece cut obliquely at the level represented by the dotted line in Fig. 27. Twelve pieces composed the series of which this was one. After about five weeks it was noticed that eye-spots were present in this one piece, and also that the amount of new tissue was greater than in any other pieces of the series. Ten days later (forty-eight days after section) the piece had attained the condition represented in Fig. 42. No other similar case has ever been observed. The regenerative power of this piece was as great as that of the best pieces cut immediately behind the ganglia (A, Series 71, Fig. 18). As regards the motor activity of this piece during early stages, I can say nothing, since the pieces of the series were not examined individually and compared, but after the piece had reached the stage shown in Fig. 42 its motor activity was distinctly more complete than that of the other pieces of the series. It was capable of more rapid progression and the margins of the new tissue were used to some extent like those of a head. Fifty-eight days after section the piece had attained the condition of Fig. 43; the new tissue had increased still further in amount and the motor activity of the piece, especially that of the new tissue, was greater than before. Beneath the large group of eyes a small light area, probably regenerating ganglia, was observed. It is very evident that in this case there is an approach to regeneration of a head. The new tissue is used as a head though in less degree than in the normal animal. It is impossible to assign a definite reason for the occurrence of this single case. In all my experiments nothing of the sort has ever been observed in other pieces at this level. Moreover, this piece shows a degree of regeneration as great as the best cases from levels immediately posterior to the ganglia (A, Series 71, Fig. 18). Whether complete regeneration of the head would have occurred could not be determined. Seventy-eight days after section the piece died without having advanced perceptibly beyond the stage of Fig. 43. Possibly if the piece could have been fed complete regeneration might have taken place. At all events this case is of great interest, since it indicates that under certain conditions the amount of regeneration at a given level may be much greater than the usual amount. Whether



the result in this case was due to some difference in the distribution or arrangement of nervous structures, to exceptional vigor, or to an exceptional amount of reserve energy it is impossible to determine. The parallelism between motor activity and regenerative power is also well shown in this piece.

##### 5. *Anterior Regeneration After Repeated Section.*

In his work upon *Leptoplana atomata* Schultz ('02) reached certain conclusions widely different from my own with respect to anterior regeneration, yet the results of experiments described by him are essentially similar to my own as far as they go. As will appear, Schultz's conclusions are probably due to the fact that his experiments were confined to a particular region of the body. He was never able to obtain anterior regeneration "selbst bei solchen Exemplaren nicht, denen nur ein geringer vorderer Körperabschnitt, weit vor dem Pharynx, abgeschnitten wurde." The position of the cut with respect to the cephalic ganglia in such cases is not stated, but there can be little doubt that it was posterior to the ganglia. In fact, it is probable that Schultz never observed anterior regeneration in the presence of the cephalic ganglia. I have no doubt that if he had done so he would have obtained results similar to my own and would have reached entirely different conclusions regarding the absence of anterior regeneration. His views on this subject are briefly stated as follows: the margins of the cut come together in such manner that union occurs and thus the region where regeneration would begin ("Regenerationspunkt") is separated from the periphery by old tissue and, moreover, the union of the muscular layers over it prevents further growth.

This attempt at a simple explanation of the absence of anterior regeneration involves, I believe, a complete inversion of the actual course of events. The cut edges unite because there is not sufficient new tissue formed to prevent this union, which is probably due to mechanical conditions in the tissues. It is very probable, however, that after their union the outgrowth of new tissue from the end cannot occur. The multiplication of parenchyma cells which Schultz describes as occurring within the muscular layer



would probably, if exposed, not produce anything like complete anterior regeneration except in the presence of the cephalic ganglia. It is of course possible that differences between *L. atomata* and *L. tremellaris* may exist; for example, in the former the production of new tissue at the anterior end in the absence of the cephalic ganglia may be so slow that union of the sides of the cut occurs before its amount is appreciable, while in the latter sufficient new tissue is formed to prevent complete union of the old cut edges. It is extremely improbable, however, that regeneration of the region anterior to the cephalic ganglia does not occur. Even in the rhabdocœla, most of which have little power of regeneration, the preganglionic region is regenerated rapidly and completely. The experiments already described are, I think, sufficient to show that no such simple explanation as that of Schultz's will suffice for *L. tremellaris*. Certain other experiments which I performed with this particular purpose in view may be described since they afford some facts of interest.

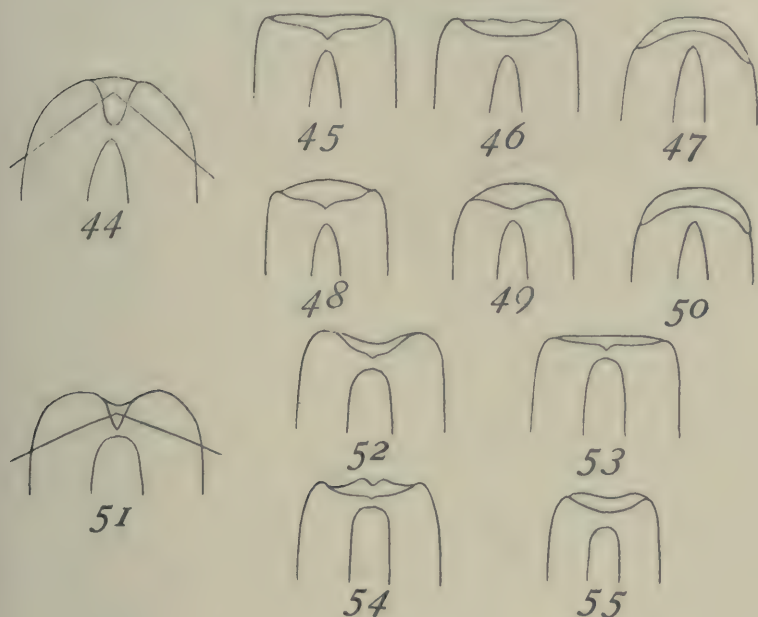
In these experiments posterior pieces from various levels were employed, but chiefly those from the pharyngeal region, since contraction of the cut surface is most marked here (see Figs. 38 and 39). After section such pieces were allowed to remain undisturbed during several days in order that the cut surface might be contracted as much as possible. Then they were subjected to a second operation, in which the lateral regions which had been drawn toward the median plane by the contraction were removed.

Two series of this kind from different levels are described; these were begun at the same time and examined at the same intervals and so are available for comparison as to the amount of regeneration at different levels after a second operation.

*Series 75.* Five posterior pieces were obtained by cuts just anterior to the anterior end of the pharynx.

Eight days after section all were either like Fig. 44 or somewhat more widely open, the space being filled with new tissue. All were cut in the manner indicated in Fig. 44. Contraction after this second operation was comparatively slight and the anterior cut surface remained widely open in every case. The condition

of the pieces ten days later is indicated in Figs. 45, 46 and 47. In spite of the fact that there was no trace of any physical obstacle to growth, such as Schultz believes the closure of the cut surface constitutes, little regeneration has occurred. The condition of the pieces a month after the second operation is indicated in Figs. 48, 49 and 50. The new tissue has increased slightly in amount but although there is no union of the old muscular layers on the



cut surface no head has been formed. After another month no further change except reduction in size had occurred.

*Series 76.* Five posterior pieces were obtained by a transverse cut through the middle of the pharyngeal region. Eight days after section all resembled Fig. 51, the contraction of the cut surface being in some cases somewhat greater, in others less. At this time the anterior part of the contracted lateral regions was removed in the manner indicated by the lines in Fig. 51.

Figs. 52, 53 and 54 represent the condition of the pieces ten days after the second operation. The double outgrowth in Fig.

54 is of interest; growth was probably more rapid in the lateral regions in consequence of the presence of nerve cords and perhaps also because of the presence of the pharyngeal pouch and the almost complete absence of parenchyma in the median line. A similar duplication was observed in a number of cases of this kind, but was in all cases only temporary, disappearing completely in later stages.

A month after the second operation all five of the pieces presented about the condition shown in Fig. 55, the differences being slight. No further regeneration occurred.

Here, as in Series 75, there is no physical obstacle to growth, such as Schultz believes to exist, yet regeneration of the head does not occur.

These two series show clearly that the explanation of the absence of anterior regeneration given by Schultz for *L. atomata* certainly does not apply to *L. tremellaris*. There is little doubt that the conditions in *L. atomata* are similar. Apparently the cut surface in *L. atomata* contracts to a greater extent after section than that of *L. tremellaris* but repeated section in the manner described would undoubtedly leave open cut surfaces sufficient for the occurrence of regeneration.

These two series are also of interest in connection with the question discussed in the preceding section, viz: the relation between the level from which regeneration occurs and the amount of regeneration. The level of the anterior ends in Series 75 is just anterior to the pharynx while in Series 76 it is the middle of the pharyngeal region, *i. e.*, much further posterior. At the time of the second operation, eight days after the first, the amount of regeneration is greater in Series 75 (Fig. 44) than in Series 76 (Fig. 51). Ten days after the second operation a similar difference between the two series exists (compare Figs. 45-47 with Figs. 52-54) and the same is true of the final stages a month later (compare Figs. 48-50 with Fig. 55). As regards motor activity a distinct difference between the two series was noted, Series 75 being the more active and capable of somewhat more coördinated movement. The differences in activity between the two series were noticeable, chiefly in the later stages. These facts agree with



the others presented in the earlier sections and show that the degree of contraction of the cut surface is not the determining factor as regards the amount of regeneration, but that both the contraction and the regeneration are determined by other factors certain of which differ qualitatively according to the level.

6. *Résumé of the Experiments on Anterior Regeneration.*

The preceding sections have established several facts of importance regarding anterior regeneration. It has been found that at all levels anterior to the middle of the cephalic ganglia anterior regeneration is complete and its rapidity is in general proportional to the size of the part removed. At all levels posterior to the middle of the cephalic ganglia anterior regeneration is incomplete and the rapidity and amount of regeneration are in general inversely proportional to the size of the part removed, *i. e.*, the further posterior the level from which regeneration occurs the less the rapidity and amount of regeneration. Complete regeneration of the cephalic ganglia is possible when no more than half the ganglionic material is removed, but never occurs after the removal of a larger part or all of the ganglionic material. When the ganglia are absent, or if they fail to regenerate completely, nothing that can properly be called a head is regenerated. A close parallelism between the degree of motor activity and the power of regeneration exists in all cases. The relation between the cephalic ganglia and anterior regeneration is very different from that between the ganglia and posterior regeneration (Child, '04b). Posterior regeneration is qualitatively complete, though somewhat reduced in amount in the absence of the ganglia while under similar conditions anterior regeneration is both qualitatively and quantitatively incomplete in high degree. The correlation between the ganglia and posterior regeneration, which is merely quantitative, was interpreted in the preceding paper as essentially functional, the motor activity of the parts being much greater though not widely different qualitatively when the ganglia are present. As regards anterior regeneration the case is different; the motor activity of this region of the body is not only less in degree in the absence of the ganglia but it is different in kind.



Since the head, like other parts of *Leptoplana*, is in large degree a complex organ of locomotion, we find that motor activity is an important factor, perhaps the most important in the formation of a characteristic "head." The anterior end does not show the characteristic activity of a "head" in the absence of the ganglia and no head is regenerated. On the other hand, in all cases where a sufficient amount of the ganglionic tissue remains intact to permit the continuance of the characteristic functional activity the regeneration is both qualitatively and quantitatively complete and forms a "head." In short a "head" is regenerated at the anterior end when this part of the body functions in the manner characteristic of a head. The relation between the indeterminate anterior regeneration in the absence of the ganglia and motor activity is indicated by the parallelism between regeneration and motor activity at different levels and from this results the inverse proportion between the amount of material removed and the amount of anterior regeneration from levels posterior to the ganglia.

We may conclude then that in the presence of cephalic ganglia a characteristic head must develop since the ganglia determine the functional relations of the various parts and so a characteristic structure results. But why do not the ganglia themselves regenerate after they are completely removed? Briefly stated the answer is this: because the other parts of the nervous system do not give rise to the functional conditions necessary for formation of the ganglia.

The first stage in anterior regeneration is, as in the case of posterior regeneration, the appearance of new tissue at the cut surface (see Child '04b). The appearance of this tissue is doubtless the result of the altered conditions in this region and is in part a rearrangement of old material in consequence of altered mechanical conditions and in part a proliferation. This material is without doubt capable of forming any region of the body, for if it arose from a posterior cut surface at the same level it would regenerate into a posterior end. Its fate is a function of its position, if we interpret position as not simply space-relation but functional correlation with other parts. In other words the new

tissue develops according to the manner in which it is used by the animal or piece. In the presence of the cephalic ganglia or a sufficiently large portion of the ganglionic tissue the anterior end of the piece is used in a characteristic manner and this functioning must subject the new tissue to a characteristic complex of conditions. It is not my purpose to attempt a description or enumeration of all the possible factors concerned in the differentiation of the parts; such an attempt would be at present in large part a series of surmises. Among these factors, however, are functional nerve stimuli, chemical and physical conditions in the tissues resulting from them, all the complex of metabolic factors as influenced by the particular conditions, and the conditions, mechanical and otherwise, connected with and resulting from motor activity or attempts at such activity.

The relative importance of different factors differs widely in different cases. In the present case the motor activity determined by the presence or absence of the ganglia is of great importance. It is only on this basis that we can explain the fact that anterior to the ganglia the amount and rapidity of regeneration are directly proportional to the amount of material removed. We have, I think, no adequate conception of the complex interactions leading to the formation of such a structure as the head of *Leptoplana*, but that motor activity is an important factor, mechanically and perhaps otherwise, cannot be denied. The constantly varying physical conditions resulting from motor activity may seem incapable of giving rise to any definite or characteristic form, but the important fact is that, notwithstanding their constant changes, they constitute a characteristic series frequently repeated. From the time when the first traces of motor activity appear in the new tissue up to complete development the animal is using or attempting to use this part in a definite characteristic manner. The arrangement of muscles and the nerve connections are of course directly responsible for this characteristic motor activity, but the characteristic arrangement of these structures can be accounted for only by other characteristic functional activities and their correlations, and so on. We are led finally to a protoplasm possessing certain elementary and characteristic activities, but that any substances

representing in themselves a "head" or other morphological features are present it is difficult to believe. The head of *Leptoplana*, like other parts of the body, represents the effect of a series of characteristic activities upon a given complex of substances in a given environment.

#### B. LATERAL REGENERATION.

Only a brief consideration of the phenomena of lateral regeneration is necessary since the relations are not essentially different from those already described for anterior and posterior regeneration. In general the amount and rapidity of lateral regeneration in the presence of the cephalic ganglia are directly proportional to the amount of tissue removed. In the absence of the ganglia the amount and rapidity of regeneration differ in different cases; if the size of the part removed is not great the amount and rapidity of regeneration are directly proportional to it; if on the other hand the portions removed constitute the greater part of the body the amount and rapidity of regeneration are inversely proportional to the size of the part removed. Qualitatively, however, lateral regeneration resembles in most respects posterior regeneration, in that absence of the cephalic ganglia does not alter the structural character of the regenerated tissue except in the extreme anterior region, though it does reduce its amount.

As in the case of anterior and posterior regeneration it was found that when about half or more of the ganglionic tissue was present complete regeneration occurred, but when less than this amount was present the results approached those obtained from pieces without ganglia. Specimens separated into halves along the median line, each half containing one-half of the ganglionic mass, were capable of complete regeneration (Child, '04a). If, however, the plane of section did not coincide with the median plane complete regeneration occurred only in the piece containing the larger part of the ganglionic mass.

As might be expected the relation between the cephalic ganglia and motor activity is the same in pieces cut longitudinally as in other pieces. When half or more of the ganglionic substance is present the pieces behave essentially like normal animals, but



when less than half the ganglionic tissue remains their behavior approaches that of pieces without ganglia. Moreover, if after removal of the ganglia by a transverse cut the body be split longitudinally or near the median plane the two halves show similar activity which is not very different from that before their separation; if, however, the plane of section lies far to one side of the median plane the smaller piece shows scarcely any motor activity, while the longer retains the characteristic activity of a piece without ganglia. These differences are of course, due to the nervous system. When the plane of section lies near the median plane each half contains the nervous structures of that half of the body, but as the plane of section approaches the lateral margin the smaller pieces contain less and less of the nervous system until in the extreme lateral region nothing remains in them but some of the peripheral nerves. Probably closer observation would show rapid changes in motor activity according as the plane of section passed on one side or the other of one of the nerve cords, but I have not paid especial attention to this point.

Comparison of this description of the motor activity of longitudinally cut specimens with the statements regarding lateral regeneration renders it clear at once that the same parallelism between motor activity and regeneration exists as in the cases already discussed, so that nothing is to be gained by going over the whole series of experiments upon lateral regeneration. I shall describe only a few cases by way of illustration, calling attention to certain points of especial interest.

Cases of the removal of less than half the body by longitudinal section from end to end need not detain us since in all cases the amount and rapidity of regeneration vary with the size of the part removed, *i. e.*, the larger the part removed the more rapid the regeneration, so that regeneration of a large part does not require much more time than regeneration of a small part. These relations are similar to those described for posterior regeneration from levels posterior to the ganglia and for anterior regeneration in the presence of the ganglia, and are to be interpreted in the same manner as due to the rôle which the new tissue is required to play in functional—doubtless chiefly motor activity.



Regeneration after longitudinal section near the median plane and regeneration of small lateral pieces present some features of interest; a description of cases of each kind is accordingly given.

*Series 64.* A large specimen was sectioned longitudinally slightly to the left of the median plane (Fig. 56). The plane of section passed through the left cephalic ganglion, leaving only a small portion of the ganglion together with most of the eyes in the left piece. After section both pieces underwent considerable contraction, but the left much more than the right. The two pieces differed widely as regards behavior; the right piece, containing nearly all the ganglionic tissue, behaved like a normal animal, advancing rapidly and using the margin of the head in the characteristic manner. The left piece, on the other hand, behaved essentially like a piece without ganglia. It never became extended, did not adhere closely to the substratum, all movements were slow and the head region showed none of the characteristic motor activity of normal animals. The pieces were examined at intervals of from seven to ten days.

The most conspicuous difference between the two pieces is the difference in form (compare Figs. 57 and 58). The right piece is much contracted and bent toward the left by the reduction of the cut surface, but the left piece is contracted into almost circular form with the cut surface greatly reduced. Both pieces move in circles in consequence of the form but the radius of the circles described by the right piece is much greater than in the left piece, which simply revolves in a space scarcely greater than its own diameter.

The difference in form and consequently the difference in direction of movement is of course the direct result of the widely different degree of contraction in the two pieces; and this difference in contraction is undoubtedly due in large part if not wholly to the differences in motor activity in the two pieces; and finally the differences in motor activity depend essentially upon the distribution of the tissue of the cephalic ganglia between the two pieces. The right piece creeps about like a normal animal holding to the substratum by means of the margin and posterior end; thus the body of this piece is subjected to the characteristic longitudinal

tension resulting from progressive movement (Child, '04a) which antagonizes the conditions at the cut surface. Thus, as the animal advances, the cut surface is frequently stretched to a considerable extent. The position represented in Fig. 57 is between the two extremes and represents the usual form during undisturbed progression. In the rapid progressive movements following strong stimulation the body often becomes almost straight and on the other hand when at rest or moving very slowly is more curved than in the figure.

In the left piece, however, rapid progression does not occur and the body adheres only very slightly; consequently the body is not subjected to longitudinal tension and the cut surface is not stretched but continues to contract indefinitely, like the anterior cut surface in pieces without ganglia. These cases only confirm the opinion already expressed that ordinary muscular contraction has little or nothing to do with the contraction of cut surfaces; it may be that muscles which have been cut and are thus free at one end gradually shorten, but this process is by no means the same as muscular contraction in the ordinary sense. Even after long periods the animals are unable voluntarily to straighten their bodies; nothing but mechanical tension in the longitudinal direction will accomplish this end—and this fact is one of the most striking proofs of the influence of mechanical tension upon form in these animals. As has been suggested elsewhere the contraction is probably due to surface tension, capillarity, elasticity and other mechanical factors, which are visibly effective in this way only in the absence of the mechanical conditions which under normal circumstances antagonize them. Doubtless also the reduction in functional activity of the parts is accompanied by more or less reduction in size.

Comparing now the regeneration of the two pieces it is evident that it is much greater in amount in the right piece (Fig. 57) than in the left (Fig. 58). The thickness of the new tissue is about the same in both, but in the right piece its width and length are both greater than in the left piece. Intestinal branches are growing, out into the new tissue anterior to the pharynx in the right piece, but none are visible in the left piece. The new tissue, especially

its anterior portion, was used by the right piece in movement, while in the left no such use was observed.

*Forty-six Days after Section.* (Figs. 59 and 60.) The two pieces present the same differences in form and activity as before. In the right piece (Fig. 59) the amount of new tissue is much greater than before, especially anterior to the pharynx, and the intestinal branches have grown well out into the new tissue in the anterior region and to a less extent in the region posterior to the pharynx. In the left piece, on the other hand, the changes are only slight. The amount of new tissue is scarcely greater than before, and the intestinal branches extend only a very short distance into the anterior region and are not visible elsewhere.

*One Hundred and Six Days after Section.* (Figs. 61 and 62.) In the right piece (Fig. 61) the relative amount of new tissue has continued to increase and regeneration of the left ganglion and the left side of the pharynx is complete. The width of the new tissue is greatest anterior to the pharynx undoubtedly in consequence of the greater motor activity of this part and in this region the intestinal branches extend out to the margin and at the anterior end of the pharynx some branches extend posteriorly. Posterior to the pharynx intestinal branches are also present though they do not fill the new tissue so completely, and some of them extend anteriorly at the posterior end of the pharynx. No intestinal branches are present on the left side of the pharyngeal region except those that extend into it from around the two ends. The posterior end of this piece is now almost straight (compare Fig. 57); it has been subjected more frequently and in greater degree than the other parts to longitudinal tension, since it is the chief organ of attachment.

The left piece, on the other hand, has not changed greatly. It is possible that some degree of regeneration of the cephalic ganglia has occurred, though new ganglia are not clearly visible; at any rate more or less extension often occurs and results in bringing the piece into the form shown in Fig. 62. The physical conditions of the old tissues near the cut surface have gradually changed during contraction and the new tissue, having arisen while the piece was contracted, is capable of much less active extension than the old



parts, for the latter were originally much more extended and have not lost that power completely. When the piece extends these new parts cannot stretch as far as the old and consequently the piece assumes the spiral form shown in Fig. 62. This is the nearest approach to straightening that is possible in this piece. In this condition the piece simply creeps over itself in the direction of the arrow. As the piece becomes quiet it gradually assumes the form of Fig. 60, though it is of course smaller than at that stage. Regeneration in this piece has not advanced perceptibly during the two months since the stage of Fig. 60.

When we compare the two pieces it is evident that although the amount of material removed was almost exactly the same in both yet the results are very different. Here, as in other cases, the only satisfactory interpretation of the difference in results is that which regards them as the consequence of the differences in functional activity of the two pieces.

The results of longitudinal section near the median plane were examined in numerous other series. In every case where one piece contained most of the ganglionic tissue and the other only a small portion the results were similar to those just described. In cases where the plane of section was nearer the median plane the difference between the two pieces was not as great and the piece containing the smaller part of the ganglia regenerated the missing parts more or less completely, and in all such cases the motor activity and general regenerative power approached that of the other piece as the part of the ganglia remaining became larger.

In a few cases I succeeded in making the section so near to the median plane that both pieces contained approximately equal parts of the cephalic ganglia. Both behaved essentially like normal animals and regenerated completely. Other cases of this sort are described in another connection in an earlier paper (Child, '04a).

In all pieces capable of complete regeneration, like the right piece in Series 64, certain features of interest were noted. First, the new tissue in the region anterior to the pharynx regenerated more rapidly than that in other regions and finally became wider than at any other point (Figs. 57, 59, 61); second, in this region the



intestinal branches grew into the new tissue more rapidly than elsewhere and finally filled it almost as completely as in a normal animal; some branches also extended from this region posteriorly into the pharyngeal region; third, intestinal branches also appeared in the new tissue posterior to the pharyngeal region but somewhat later than those in the head region; these never attained so great an extent as those in the anterior region but some extended anteriorly into the pharyngeal region.

These three features were, as stated, characteristic of all pieces of this kind and must therefore possess a certain significance. The first two, viz: the more rapid regeneration of the lateral regions of the head and the more rapid growth of the intestinal branches in this region are connected. In my opinion both are due to the fact that this region shows the greatest motor activity of any part of the body. Its characteristic activities have already been described (Child, '04a). Characteristic conditions of tension and pressure result from these characteristic movements and these are undoubtedly factors in the arrangement of the material and so in determining the form and may themselves constitute stimuli to growth. As regards the intestinal branches the internal pressure due to intestinal contents must undergo change with the movements as the fluid is forced into or out of the branches as contraction or extension occurs. In the head-region the internal pressure in the peripheral branches of the intestine is greater than in other parts, *i. e.*, the contents are forced into these branches more frequently and probably also with greater pressure than elsewhere. Brief observation of a specimen with well-filled intestine is sufficient to demonstrate these facts very clearly. There can be no doubt that these conditions of internal pressure play a part in the development of the intestine; some experimental evidence upon this point has been obtained from study of another species; this I hope to present at another time. Admitting that the internal pressure is a factor in the development of the intestine it is easy to see that the same conditions, viz: greater functional motor activity, which bring about the more rapid regeneration in the anterior region also bring about the more rapid growth of the intestinal branches in this region.

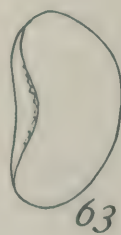
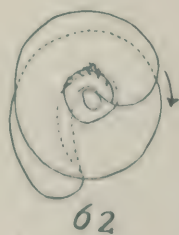
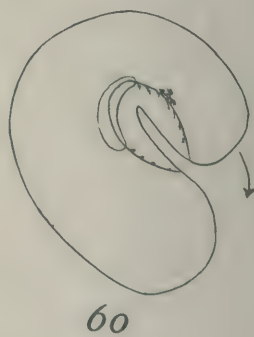
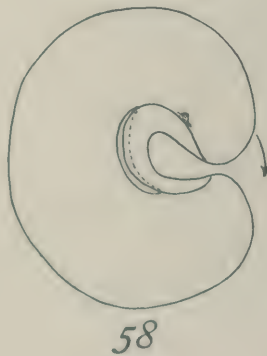
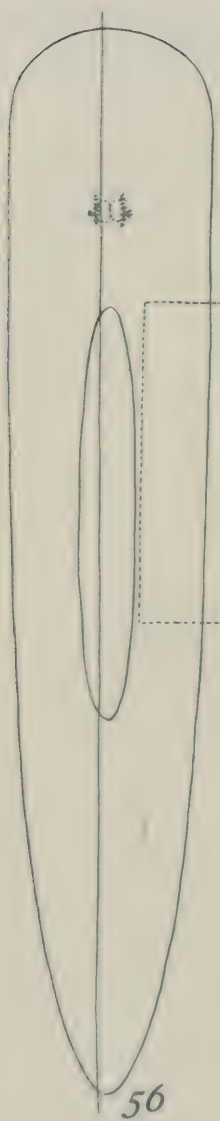
The region posterior to the pharynx does not show as great a degree of motor activity as the anterior region and we find correspondingly that the development of the intestinal branches is less rapid here than there.

The growth of intestinal branches in a postero-lateral direction from the pharyngeal region and in an antero-lateral direction from the region posterior to the pharynx is also of much interest. In none of these pieces did lateral intestinal branches regenerate from the pharyngeal region.

We may regard the branched form of the polyclad intestine as primarily the result of distension by internal pressure of a sac surrounded by parenchymatous tissue. The distension occurs along paths of least resistance and greatest internal pressure. Its direction is the result of several factors, viz: the direction of internal pressure, the position of paths of least resistance, and the presence or absence of antagonistic external pressure, however produced.

Since the internal pressure in each branch is transferred in some degree to the tissues about it this pressure must constitute for an adjoining branch external pressure antagonizing the internal pressure in the second branch and so preventing its extension toward the first. In short, intestinal branches cannot enter a region already occupied by such branches unless the internal pressure in the former is much greater than in the latter.

In the case under consideration at present, viz: the growth of intestinal branches in "abnormal" directions from the regions anterior and posterior to the pharynx into the pharyngeal regions, I believe that the absence of intestinal branches in this region is the determining condition. The intestinal contents, being forced into the branches anterior and posterior to the pharynx, exert pressure upon the walls of these and growth occurs along the lines of least resistance. Under ordinary conditions the presence of other similar branches would in consequence of the pressure upon the tissues prevent the growth of branches postero-laterally in the one case and antero-laterally in the other (Fig. 61). Here, however, such branches are absent and consequently the mechanical resistance to extension of the branches in this direction is not





greater than elsewhere; hence the "abnormal" or as it might be called "regulative" distribution of these branches.

But the absence of intestinal branches in the pharyngeal region in these pieces is itself a fact of importance. Though I was unable to determine with certainty the particular conditions to which this absence of regeneration is due certain points which appeared to me suggestive may be mentioned here. First of all it must be remembered that in the absence of food the intestinal contents decrease in amount during the course of these experiments and consequently internal pressure diminishes; the movements and muscular contractions force the contents into one part or another of the intestine thus producing distension now in one region, now in another; secondly, the regions of greatest muscular activity are the regions anterior and posterior to the pharynx, especially the former; and it is in these regions that the movements of the intestinal fluid are visibly most frequent and powerful. The lateral margin of the body in the middle region shows much less motor activity than the terminal regions and in pieces in which the longitudinal axis is bent by contraction of one side this difference is especially marked on the concave side. And finally, when contraction of the longitudinal muscles occurs and the intestinal contents are forced back toward the pharyngeal region the concave side of the body becomes still more concave and the new tissue of this side is pressed together and often thrown into folds, a condition which must retard or prevent the growth of intestinal branches into this region. In short, I am inclined to believe that the form of the piece and the distribution by means of muscular activity of internal pressure due to intestinal contents are the chief factors in preventing regeneration of the intestinal branches in the pharyngeal region.

In all cases where the plane of section is far to one side of the median plane the amount of regeneration is even less than in the left piece of Series 64. Moreover, as the distance between the median plane and the plane of section increases the rapidity and amount of regeneration decreases and the life of the pieces becomes shorter; narrow strips from the lateral regions of the body living only a few days and showing scarcely any regeneration.

*Series 74.* Seven pieces were cut from the region lateral to the pharynx (see dotted lines, Fig. 56). Figs. 63 and 64 show the condition of these pieces eighteen days after section. All are much contracted and are wholly incapable of movement. Some new tissue has appeared on the cut surface but it is not visibly differentiated; an irregular network of intestinal branches protrudes a short distance into the new tissue.

No further regeneration occurred and two weeks later all the pieces were dead.

### C. GENERAL CONSIDERATIONS.

According to the conclusions reached in the preceding paper (Child, '04b) posterior regeneration from levels posterior to the ganglia is affected only quantitatively by the removal of the ganglia, *i. e.*, it is reduced in amount but is qualitatively complete; in all cases the amount and rapidity of regeneration are directly proportional to the size of the part removed. Anterior regeneration on the other hand, is both qualitatively and quantitatively incomplete in the absence of the ganglia and the amount of regeneration is inversely proportional to the size of the part removed. When the ganglia are present, however, *i. e.*, at levels anterior to the ganglia the amount and rapidity of regeneration is directly proportional to the size of the part removed.

In the case of lateral regeneration the amount and rapidity of regeneration are directly proportional to the size of the part removed when the ganglia are present; when the ganglia are absent amount and rapidity of lateral regeneration in the head region are inversely proportional to the size of the part removed, while farther posteriorly the proportion is direct unless the part removed is very large, when it becomes inverse in consequence of the small size of the piece and the unfavorable conditions for continued existence.

Posterior regeneration and lateral regeneration posterior to the head region are affected only quantitatively by the presence or absence of the ganglia, but the influence of the ganglia on anterior or lateral regeneration in the head-region is qualitative as well as quantitative.

Pieces from the region anterior to the ganglia, those from the extreme lateral margins of the body and those from the extreme posterior end are almost incapable of regeneration and live but a short time.

In cases like that of *Leptoplana* where regeneration takes place by the differentiation in a particular manner of new tissue formed upon a cut surface it is very evident that the fate of the new tissue is determined primarily by its relation to the old. The old part is already definitely organized and functions in a particular manner, thus determining the conditions to which the new tissue is subjected. Attention has been called repeatedly to this relation in the description and discussion of the experiments, and a close parallelism between the amount and kind of motor activity and the amount and rapidity of regeneration and the morphological character of the regenerated part has been shown to exist. This relation between motor activity and regeneration is undoubtedly complex in character and requires some consideration.

In the preceding paper (Child, '04b) the question of the relation between the nervous system and morphogenesis was briefly discussed and attention was called to the possibility that an apparent relation may exist in cases where the nervous stimuli themselves are not the formative factors but merely determine the functional conditions. Now that the description of the experiments is completed it is desirable to return to this question and consider it in the light of the experimental data. Three possibilities must be considered: First among these is the question as to whether nervous stimuli themselves exercise a "trophic" or a formative influence. The experiments leave no room for doubt that some considerable portion of the central nervous system must be present in pieces from adult specimens of *Leptoplana* in order that life may continue. Small anterior, lateral, and posterior pieces containing only peripheral branches from the ganglia or cords die within a few days. These facts may be regarded as indicating that the nervous system is necessary for continued existence. But since neither the ganglia nor the other parts which constitute a head regenerate after removal of the ganglia we must conclude either that the influence of the nervous system is not sufficient in



itself for complete regeneration or else that different influences are localized in different parts of the nervous system. The fact that posterior regeneration is qualitatively complete in the absence of the ganglia while anterior regeneration is not might seem to favor the second alternative. Even if we admit that nervous stimuli themselves exert a formative influence there seems to be no good reason for supposing that this influence is due to stimuli of a particular kind differing from other nervous stimuli. Indeed the general consensus of opinion is that in all probability a given nerve cannot transmit qualitatively different stimuli. The question of the direct formative influence of nervous stimuli has been much debated; in all cases, however, where there is a possibility of the existence of such an influence distinction between it and the formative effect of the functional conditions cannot be made experimentally.

The second possibility to be considered is the formative effect of functional conditions which plays so important a part in the views of Roux ('95) and others. According to these views the conditions connected with functional activity of a part exert an influence which may produce growth or further development of the part. The functional conditions may be various in kind—either physical or chemical. In the organs of movement and support their supposed formative influence has been most clearly recognized. If functional conditions exert a formative effect it is clear, as Roux has pointed out, that in those organs whose function is determined by nervous stimuli a formative influence of nervous stimuli will seem to exist since the functional conditions are determined by the existence of a connection between the organs in question and the nervous system. In reality, however, this relation between the nervous system and formative influence is indirect (see Child, '04b, p. 507), since not the nervous stimulus itself but the change brought about in the organ and its environment by function are the essential factors.

In a paper which has recently appeared Goldstein ('04), after a consideration of the influence of the nervous system upon embryonic development and regeneration, reaches the conclusion that the functional stimuli and not the nervous stimuli themselves

are the important factors in cases where any relation exists. His statement of the case is as follows: "Diejenigen Organe, deren Funktion wesentlich durch den anatomischen Zusammenhang mit dem Zentralnervensysteme (durch Nerven) vermittelt wird—und um ein solches handelt es sich bei der Muskulatur—bedürfen zu ihrer Bildung sowohl als zu ihrer Regeneration in schon relativ früher Periode der Entwicklung am notwendigsten der Verbindung mit dem Zentralorgan, wie auch ihre Erhaltung und Regeneration in postembryonaler Zeit unbedingt an diese Verbindung gebunden ist. Dagegen sind diejenigen Organsysteme, deren Funktion wesentlich durch den Einfluss der unmittelbaren Umgebung bedingt ist, wie der Knochen, unabhängig vom Zentralnervensystem sowohl in ihrer Entwicklung (resp. Erhaltung) als ihrer Regeneration, soweit durch eine eventuelle Unterbrechung der nervösen Verbindung die normale Tätigkeit der Umgebung nicht gleichzeitig beeinträchtigt wird (z. B. Störung der Funktion des Knochens durch Aufhebung des Tonus der Muskulatur durch die Nervendurchschneidung) und das betreffende Organ, also zum Beispiel der Knochen, auch sonst in seinen normalen Beziehungen zur Umgebung erhalten wird."

This paper which is, I think, one of the most satisfactory contributions to the subject which have appeared includes much literature of which various other recent writers on this subject, notably Herbst ('01) Wolff ('02) and Moszkowski ('03) appear to be ignorant. The criticism of the remarkable conclusions of Herbst is of much interest and must be regarded as effective. The work of Harrison ('01, '03), Bardeen ('00), and other authors which demonstrates clearly enough that the voluntary muscles arise before nervous connections are established is given full consideration; for a bibliography and critical examination of these and other points the reader is referred to Goldstein's paper.

That regeneration and embryonic development may differ as regards relative importance of the nervous system is also admitted by Goldstein, but, I think, the importance of the relation in regeneration between the new part and the old completely differentiated part with its characteristic functional activity is not clearly recognized. In very many cases of regeneration motor activity of a

part appears long before the characteristic form is established. In *Leptoplana*, for example, when the head is cut off just anterior to the ganglia, or in *Planaria* at any level, the new tissue growing out from the cut surface begins to function in the manner characteristic of the head long before it has acquired the form of a head. All the facts indicate that the definitive form is the result of this activity. Here the indirect influence of the nervous system is a necessary factor in the result. Only the earliest outgrowth of tissue before motor activity appears is probably due in large part or wholly to the stimulus of the wound and altered local conditions.

If we attempt to interpret the close parallelism between motor activity and regeneration in *Leptoplana* in accordance with the hypothesis proposed above we may suppose that the motor activity exercises a formative effect upon the regenerating part and so determines its fate. But in order that coördinated motor activity may occur in a given part some degree of differentiation into contractile and conducting structures must exist. We may regard the regeneration of particular parts of the nervous system in the new tissue as dependent primarily upon functional stimuli proceeding from the old, already organized part of the nervous system. In this relation we have a case where the nervous stimulus and the functional stimulus are identical.

The muscles in the new part may develop under the influence, direct or indirect, of stimuli proceeding from the nervous system, though it must be borne in mind that visible morphological connection between these parts and the nervous system may not be present. In certain other cases, however, as in that of the legs of *Triton* described by Wolff ('02) and discussed by Goldstein, the definitive form is apparently independent of the characteristic motor activity, though in these cases the physical conditions resulting from differences in the rapidity of growth of different parts may play a rôle. The hypothesis of the physiological formative influence of function is undoubtedly important but still another possibility remains to be considered, viz: the direct mechanical effect of mechanical conditions. That the arrangement and distribution of material is in many cases the direct effect of mechanical conditions cannot be doubted.



The possibility of a mechanical interpretation of many of the phenomena of cell life has been shown by Rhumbler in various papers, and even if his application of mechanical principles should prove to be too general, the importance of mechanical factors in the cell must be recognized. If we admit the importance of these factors in the cell we cannot avoid the conclusion that their importance is certainly not less and probably much greater in multicellular and complex structures. The rapid advances of physiological chemistry have turned the attention of many workers away from the physical aspects of the problem of form to such an extent that in some quarters attempts at mechanical interpretation of organic phenomena are accepted with more or less reluctance. There can be no doubt that chemistry is almost daily throwing new light upon organic phenomena but, I think, it is no less certain that any essentially chemical theory of form must be incomplete. One reason for the somewhat helpless attitude of certain authors before the problem of form is, I am convinced, due to their failure to recognize the importance of physical as opposed to chemical factors. We may recognize the importance of these factors without making of them a universal principle of interpretation. All the present and future resources of both chemistry and physics are necessary for the interpretation of biological phenomena, but especially in connection with the problem of form in which masses and mass-relations are an important element are mechanical principles of interpretation of value.

Roux has pointed out the importance of mechanical conditions as functional stimuli, and hence, according to his view, as formative factors in the case of various organs whose function is wholly or in part mechanical. But we must admit that mechanical conditions as stimuli exert in many cases not only a more or less complex physiological influence but produce a direct mechanical effect and that this effect under typical conditions is typical. The change of form in pieces of *Stenostoma* (Child, '02, '03) in consequence of mechanical tension and the asymmetrical form of pieces of *Leptoplana* (Child, '04a) resulting from changes in the direction of locomotion are cases in point. No one would be more ready

than myself to admit that mechanical factors are not the only factors in these cases, though their importance in determining the result is evident.

In analysis it is necessary, though in practice often difficult, to distinguish sharply between mechanical conditions as functional stimuli producing a "trophic" or formative effect and mechanical conditions as direct mechanical factors determining the space relations of masses. In the cases cited there is no doubt that tension and growth are connected in one way or another. Even in regard to this point, however, there is room for the question as to whether the effect is mechanical or not.

It is probable that in many cases the effect of mechanical conditions as stimuli is widely different from the mechanical effect. The reaction to the stimulus may be an increased power of resistance to the particular mechanical conditions involved, as in the case of tendon, bone and various other structures of animals and plants. The direct mechanical effect consists in the arrangement of material in accordance with the mechanical conditions of tension and pressure. In *Leptoplana* not only the form and the direction of growth of the regenerated posterior end but the amount of regeneration and the direction of growth seem to depend to a considerable extent upon, and to correspond in direction with the mechanical tension to which the part is subjected (Child, '04a, '04b). It is difficult to determine how far this result is directly mechanical and how far it is due to mechanical stimuli to growth. I think, however, that the presence of both factors must be admitted. But in most cases the elongation of the new tissue in the direction of tension does not continue indefinitely; it gradually decreases and after a time ceases even though the mechanical conditions continue as before. Undoubtedly in many cases the differentiation of the regenerated tissues and their increased power of resistance to mechanical tension determine when the elongation shall cease. This may be regarded as somewhat similar to the effect of mechanical conditions on tendon, bone, etc. But in some cases it is possible that the definitive form is merely the condition of mechanical equilibrium. In either case the part retains approximately the same form, provided that the

mechanical conditions do not undergo marked change. If any considerable permanent change in the conditions does occur the form is gradually altered until a new equilibrium of one kind or another is established.

To sum up: the close parallelism between the amount and rapidity of regeneration and the form of the regenerated part in *Leptoplana* and the characteristic motor activity of the part may be accounted for in three different ways: nervous stimuli themselves may be regarded as formative factors; the functional conditions including mechanical conditions in particular parts which are determined by functional relation between the new and old parts may be regarded as stimuli to growth and therefore as formative factors; and lastly, mechanical conditions connected with the characteristic motor activity and other functional conditions may determine directly in greater or less degree the arrangement of material and so the form.

As has been indicated in the preceding paragraphs I believe that all of these factors are concerned to a greater or less extent. The facts regarding regeneration of the ganglia indicate that conditions obtaining in the parts of the nervous system from which regeneration occurs are the essential formative factors for the ganglia. Some suggestions regarding the probable rôle of and mechanical and non-mechanical effects of non-nervous functional conditions in determining form have been made in the preceding paragraphs. My successful attempts at experimental control of form-regulation (Child, '02, '03, '04a) have convinced me that mechanical conditions are of great importance, at least in the cases considered. While I think it probable that they will be found to be equally important in many other cases, though perhaps in widely different manner, generalization would be premature. What has been said in the present section is sufficient, I trust, to make it clear that I do not exaggerate the importance of mechanical factors in morphogenesis.

The mechanical aspects of morphogenesis have been much neglected; application of mechanical principles will undoubtedly assist us in future in the consideration of organic form. But no single principle of interpretation will lead us far in biological



science. It is necessary to recognize the interrelations of numerous and widely different factors in all organic phenomena. I believe that we have as yet no adequate conception of the complexity of these phenomena. The universal desire to generalize results in misleading schematization. The processes of organic nature are even more varied than we suspect.

#### SUMMARY.

1. Anterior regeneration in regions anterior to the middle of the ganglia is complete, and amount and rapidity of regeneration are directly proportional to the size of the part removed. From levels posterior to the middle of the ganglia regeneration is both qualitatively and quantitatively incomplete, no head being regenerated. In these cases the amount and rapidity of regeneration are inversely proportional to the size of the part removed.

2. Lateral regeneraion in the presence of the ganglia is complete; in the absence of the ganglia it is complete except in the lateral head-region and the amount and rapidity of regeneration are directly proportional to the size of the part removed if the piece is large. As the size of the piece is reduced the proportion becomes inverse.

3. As in the case of posterior regeneration, there is a close parallelism between the rapidity, amount and completeness of anterior and lateral regeneration and the characteristic motor activity of the part concerned.

4. The relation between the nervous system and regeneration in *Leptoplana* may conceivably be either direct, in that nervous stimuli themselves constitute formative factors, or indirect, in that functional conditions resulting from use of the part in a particular manner determined by its relation with the nervous system, are the formative factors properly speaking. In the latter case the formative factors may be either stimuli to growth and other changes or they may be directly mechanical. The facts seem to indicate that the relation is actually indirect except perhaps as regards the regeneration of parts of the nervous system itself; the conditions are both mechanical and non-mechanical but the former are especially important in certain respects.

5. In cases of regeneration like the present the functional relation of the regenerating part to the old is an important factor in determining the fate of the new tissue.

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# EXPERIMENTS ON POLARITY IN TUBULARIA.

BY

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WITH 5 FIGURES AND 20 TABLES.

In the discussion that has arisen in regard to the factors that determine the formation of new hydranths in *Tubularia*, the red pigment in the stem and in the current has played a conspicuous rôle. Loeb first suggested the idea in 1891 without, however, stating clearly whether he referred to the pigment in the wall or in the current, although the former appears to have been intended, as he makes no mention of a current or of pigment in the current. In his last publication, however, he argues in favor of the pigment in the current being an important factor in the regeneration of hydranths. Driesch also thought that the pigment in the circulation might have at least a quantitative relation to hydranth formation. Morgan has presented evidence which, he believes, disproves that the red pigment, as such, takes any part in the formation of the hydranth, pointing out, among other objections to the views of Loeb and of Driesch, that the pigment of the current is not absorbed, but is gathered together in the digestive cavity within the new hydranth; and Stevens has observed that this ball of pigment is later vomited forth from the mouth of the fully formed hydranth. We had hoped that our observations had laid to rest the question of the supposed rôle of the pigment in regeneration, but such appears not to be the case, since in his recent publication Loeb returns once more to the problem. For this reason it has seemed to us worth while to undertake a new series of experiments, although we think the evidence already advanced more than sufficient to disprove the view that the pigment has any direct influence on the regeneration of hydranths. While we have considered this question somewhat

fully for the reasons given above, we think, however, that our new work is of more interest in connection with the problems of polarity and heteromorphosis in *Tubularia*.

The work was carried out during the summer of 1904, while enjoying the hospitality of the Hopkins Marine Laboratory of Leland Stanford Junior University. The species of *Tubularia* that is found near Pacific Grove is *T. marina*.

It differs in some respects from the forms found at Woods Hole and at Naples, the principal difference being that it has a single hydranth at the free end of an unbranched stem attached by stolons to the rocks. In regard to its powers of regeneration, it appears to be very similar to the other two forms, the main difference being its stronger tendency to produce a stolon from an aboral cut end rather than a heteromorphic hydranth.

GENERAL ARGUMENTS THAT ARE OPPOSED TO THE SUPPOSED  
FUNCTION OF THE RED PIGMENT AS A FORMATIVE  
OR NUTRITIVE FACTOR.

The discussion of the problem that follows may be made clearer, if we review here the points that bear on the supposed function of the red pigment as a formative or nutritive factor.

*First.* The stem of *Tubularia marina* contains throughout its length two ridges standing opposite to each other and nearly meeting in the middle of the stem. These ridges are connected by strands of endoderm at more or less irregular intervals, forming a perforated partition between the two circulatory canals of the stalk. The fluid in the central cavity of the stem moves up one canal, across to the other side of the partition just below the stomach of the polyp, and down the other canal. Long lash-like flagella, belonging to the endoderm cells, keep the fluid in circulation. Occasionally granules or food particles are contained in the fluid, but ordinarily it is quite clear and its movement is consequently difficult to demonstrate.

When a piece of the stem is cut off, its ends close rapidly, and the circulation continues as before. At first the fluid contains no granules, or only an occasional one. The first changes, prepara-

tory to the formation of the new hydranth, are initiated by the breaking down of the endodermal ridges near the oral end of the piece. The cells of the ridges break up, setting free their contents in the fluid. Since these cells contain red pigment, as well as other products, the fluid becomes more or less filled with these granules, as well as with broken cells or even entire cells containing the pigment. There is nothing mysterious or obscure in regard to the source of this pigment in the fluid. It owes its origin to the degenerative changes preparatory to forming a hydranth. The ridges at the aboral end of the piece are also to some extent absorbed, but much less so than at the oral end, except in cases where an aboral hydranth is forming, when the same extensive process of absorption occurs as at the oral end. It is evident that the pigment observed in the circulation is a by-product in the regeneration of the new hydranth, and from its origin we get no suggestion of its being a substance formed for building up a new structure. It will also be observed that the position of the new hydranth, indicated by the degeneration of the endodermal ridges, is already determined when the red pigment appears in the circulation.

*Second.* As has already been stated, the pigment, along with other débris from the circulating fluid, is often collected in a ball within the forming hydranth. It appears that at this time, when the primordium of the hydranth is already laid down, the soluble parts of the broken down cells in the circulation are absorbed, presumably being digested, while the *red pigment is not absorbed but is later ejected from the mouth of the new hydranth*. Whether some part of it may not be absorbed as Loeb intimates, without however showing that the process occurs, we cannot of course state positively, but if this does happen, the amount absorbed must be inconsiderable since the entire amount appears to be ejected. Even if some of it were absorbed,—of which there is no evidence,—it would be far too small in amount to account for the pigment in the new hydranth. Loeb states that “the regeneration of a new polyp proceeds from those points where the pigment granules collect.” Our observations show that the pigment granules more often collect at the distal end of the piece



anterior to the tentacle anlagen. When they are seen within the tentacle region they are by no means evenly distributed over the surface, but occur in one or more irregular masses so slightly attached to the wall that they are often washed away by the current. These masses of pigment and other débris are sometimes carried to the basal end or to a sharp bend in the stem, remain there for a time, and later appear again at the distal end. Such being the case, the pigment granules could with difficulty be imagined to be a hydranth-forming substance.

*Third.* A microscopic examination of the pigment in the wall of the tube, and also in the circulating fluid, shows that it is composed of irregular crystalline-like red and yellow particles of various sizes and shapes. In addition there are spheres of an oily appearance that may represent reserve food material. These spheres also help to give a general yellowish tinge to the stem, especially to old stems and stolons, but the main color in regions of new growth is due to the red pigment. Where a new hydranth is developing, more pigment begins to appear in the endoderm and a proportionately larger number of small granules seems to indicate that this pigment is manufactured by the cells themselves. That the endoderm cells have this power there can be no question from the facts to be mentioned presently. Why, then, since this can be shown to be the case, imagine another wholly superfluous process to go on, viz: an absorption of red pigment from the circulating fluid?

*Fourth.* If the amount of pigment in the entire circulation be compared with the amount of new pigment in the developing hydranth, it will be found that the latter is many times in excess of the former and hence must have been formed *in situ*. This is especially noticeable in very short pieces.

*Fifth.* The amount of pigment set free by the degeneration of the ridges in the hydranth-forming region is approximately the same for short and for long pieces. Hence the relative amount in the circulation is far greater in short pieces, yet these, if moderately short, develop no faster than do longer pieces, and if very short it takes a longer time for the hydranth to regenerate. In the latter case, however, though not in the former, the problem

is complicated by another factor, namely, the reduction in the length of the hydranth-forming region, which in itself seems to involve a delay in the development.

*Sixth.* The formation of new pigment is not characteristic of the new hydranth alone; but in the development of a new stolon a large amount of new pigment is formed, and at a time when there is none present in the circulation.

We may now examine in the light of these statements the arguments which Loeb believes are in favor of the red pigment being a factor in the development of new hydranths.<sup>1</sup>

With the following statements in Loeb's paper we cannot agree:

*First.* On p. 154 Loeb states, "I found further that at that end at which a new polyp forms, the formation of this organ may be recognized beforehand from the fact that red pigment granules collect at this end in relatively great density. New pigment granules are transported by a current of fluid to this end, and evidently remain lying or stuck in the neighborhood of the cut surface. The stream of fluid is kept up by ciliary movement. I expressed at that time the opinion that such a transportation of materials by the stream of fluid toward the cut end is one of the conditions of the formation of organs in *Tubularia*, and that the polarity of the *Tubularia* stem, *i. e.*, the fact that the polyp forms earlier at the oral pole, depends on the fact that the 'organ-forming' substances (possibly the red pigment granules) collect in sufficient quantity sooner at the oral than at the aboral end." We find on the contrary that the end where the polyp is to develop begins to thin out in preparation for the formation of the polyp and the primordia of the tentacles may be laid down before the red pigment begins to accumulate. Loeb speaks of the current as passing forward, and this appears to lend support to his view; but it should not be overlooked that the current flows backward on the other side of the stem and the same argument could be

<sup>1</sup>Concerning Dynamic Conditions which contribute toward the Determination of the Morphological Polarity of Organisms. University of California Publications. Physiology, Vol. I, Nos. 16 and 17. 1904.

made to show that the aboral end should develop its polyp first, because a current also flows in this direction!

*Second.* By sticking the oral end in sand Loeb found that a larger proportion of the pieces produced aboral hydranths, and in a shorter time than when suspended freely in water. This result is cited as an argument in favor of the "assumption" that the red granules are "organ-forming substances;" but it is not clear that the experiment has any bearing on this point. Loeb appears to mean that since the red substance can not be used at the oral end, which is embedded in the sand, that, therefore, it can be used by the aboral end. If this is his meaning, it should be pointed out that the original assumption has now taken a very different form, and the polarity is accounted for not by the direction in which the current flows, but by an abundant supply of a formative or nutritive substance. With this point of view, so fundamentally different from the other, we are ready to agree, provided that the nutritive substance is something else than the red pigment. The experiment of ligating the stem, which Loeb states that he carried out "during the last summer," had already been performed by Driesch ('99) and by Morgan ('01), and the theoretical questions involved were discussed by them. Loeb states that in ligated stems, only polyps appear at the aboral end and never "roots." While this is true in general, yet occasionally a "root" develops at the aboral end in the California species of *Tubularia*. There is, however, no such sharp distinction between stem and stolon as is implied by the use of the term "root," since a stolon may produce a hydranth at its end in the same way that one is produced at the end of a stem. If the stem is ligated or has its oral end stuck into sand the stolon may produce a polyp at its distal end. A fuller discussion of this property of the stolon will be taken up in another connection.

*Third.* Loeb concludes that "the ligation of a piece cut from the stem of *Tubularia* results in the suspension of the polarity as far as regeneration is concerned." On the contrary we shall bring forward evidence to show that the polarity is altered mainly near the region where the aboral polyp has developed, and that a reversal or suspension of polarity does not extend throughout the



rest of the stem. It is true that by ligating the stem a more rapid development of the aboral polyp is brought about, as Driesch and Morgan had already reported. Even bending the stem sharply produces the same effect, as Morgan has shown. This question of the more rapid regeneration of the aboral polyp when the oral end of the piece is closed will be fully discussed below.

*Fourth.* Loeb states (p. 158), "The experiments so far described had shown that by ligaturing a piece cut from the stem of *Tubularia* one abolishes the difference between the oral and aboral poles. The question arises whether one can go farther and even reverse the polarity, *i. e.*, whether one can force the aboral cut end to form a polyp sooner than the oral end. This is in fact possible, though this phenomenon does not manifest itself with the same degree of certainty as the results of the ligature experiments just described." The experiments, which he believes demonstrate this point consist first in cutting off a piece of the stem and ligating it in the middle; then after the aboral polyp has developed, the half of the stem lying aboral to the ligature is cut out, one cut end being near the ligature (the old oral end), the other near the aboral polyp (the old aboral end, but now that the polyp has appeared the new oral end). If the latter end should produce "at least in a proportion of the cases" a polyp sooner than the old oral end, this would show, Loeb thinks, that the polarity of the piece has been reversed. He found that the old aboral end (new oral end) produced a polyp before the old oral end in ten cases and in five cases the reverse occurred. From this Loeb concludes that the polarity of the piece has been reversed. Such, however, is not the case. All that the experiment really shows is that in some pieces the conditions are more favorable for the development of the polyp at the new oral end (old aboral end), where the polarity has been changed, but the rest of the piece has retained its original polarity as in the experiments in which the oral end of the piece was buried in the sand.

*Fifth.* Loeb's summary, which follows this experiment is as follows (p. 159): "If we correlate all these observations, we get the idea that the cause of the normal polarity which appears

in the regeneration of a piece cut from the stem of a *Tubularian* is based on the condition that the circulation favors the motion of various substances in the direction from the aboral to the oral pole." There is, so far as we can see, nothing in the experiments that establishes this conclusion, and facts already known seem to controvert it.

*Sixth.* After giving an approximately correct account of the complete, *i. e.*, the circular, course of the circulation (p. 160), Loeb states, "It is now generally found that only those pieces form a new polyp which possess a circulation." Since the circulation takes place in all living pieces of all sizes, this statement can have, so far as we can see, no bearing on the problem. The further statement "that the collection of pigment granules at one cut end precedes polyp formation" gives an erroneous impression and reverses the real order of events. The statement "that the regeneration of a polyp proceeds from those points where the pigment granules collect is unproved and we believe incorrect. Whether, as stated, stems rich in pigment always form polyps sooner than those poorer in pigment is questionable. It is not improbable that a stem lacking in the usual amount of pigment is not in a vigorous condition, hence the slower development of the polyp. Furthermore, the oral end of the stem is usually less pigmented than the more aboral parts, yet pieces cut from the oral end of a stem produce hydranths sooner than pieces nearer the basal end.

We conclude from an examination of Loeb's argument that the evidence is wanting to show that the polarity in *Tubularia* is "to be referred to a process which is comparable as to its variety with a process of streaming in the direction of the aboral to the oral pole." Whether the formation of a polyp involves the using up of available substances in the stem which may involve a consequent diffusion of material toward the place where it is being utilized (because there is in consequence a smaller quantity of it there) will be discussed in our general conclusions; but this idea is fundamentally the reverse of the one maintained by Bonnet, Sachs and Goebel, *viz.*: that the movement of the circulating fluid in a given direction determines *where* the

development of an organ shall be initiated, and is therefore the cause of polarity.

#### EXPERIMENTAL RESULTS.

*Experiment 1.* The following data will serve to show for *this species*, the frequency of the occurrence of aboral polyps in pieces lying on the bottom of a glass dish containing sea water. The polyp was cut from the oral end and the base cut off at varying distances above the stolon.

In long pieces, measuring from 10 to 25 mm. or more, about 5 per cent form aboral polyps (Fig. 1); the rest form stolons or do not regenerate at all at the aboral end. When short pieces are cut off, measuring about 5 mm., the percentage of aboral hydranths (double-headed pieces) was larger, 8.5 per cent. When very short pieces are cut off the same kind of incomplete structures that have been described for the Naples and for the Woods Hole species appear, and some of them are double structures. In some cases the new head and stolon were cut off in order to see if a larger percentage of aboral heads would develop, but this did not occur. In another



FIG. 1.

series the new head only was cut off but this did not cause the aboral ends to produce hydranths; nor were they produced when only the stolon end was removed.

*Experiment 2.* In order to see what changes, if any, had been produced by the development of an aboral polyp in long pieces having an oral polyp also, the polyps at each end were cut off and the piece cut in two in the middle (Fig. 2). The pieces were

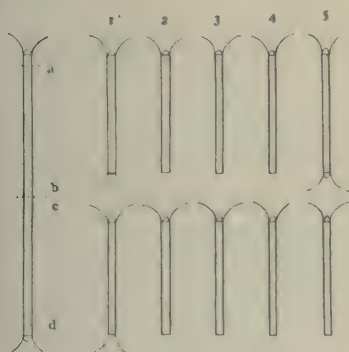


FIG. 2.

kept oriented so that the different ends were known. If the formation of an aboral polyp at *d* had the same influence on the stem as the formation of an oral polyp at *a*, the middle *b c* would be a



sort of neutral zone without any polarity or with a weak polar condition, if such a thing is possible. The results show that the original polar condition still manifests itself in this region, as seen in the following figures for five pieces (Fig. 2).

The aboral head at *b* in piece 5 can be accounted for without assuming it to be due to a change of polarity, for aboral heads, as we have seen, sometimes develop on short pieces. The absence

of a polyp at *d* on the other half indicates that the change caused by the aboral polyp is not great. Later a polyp appeared on piece 1 at *b* and at *d*.

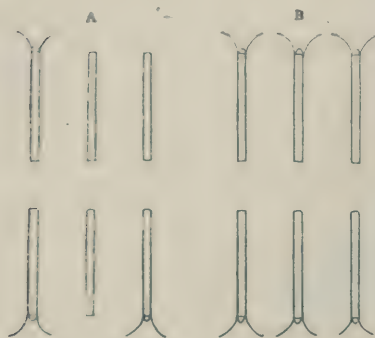


FIG. 3.

In the next series (Fig. 3), with three pieces, the influence of the aboral polyp is better seen, but the old polarity still shows itself in the oral heads. These pieces were cut June 30. Record A was taken July 2; record B, July 3.

In another series four pieces developed as shown in Fig. 4. In the first and third pieces the old and the new polarity are exhibited in the aboral half.

The following table gives the results for 19 pieces cut as above at different dates, from June 27 to July 24. The figures indicate the order in which the hydranths appeared. The letter *s* indicates a stolon.

The appearance of hydranths simultaneously at *b* and *c* and of stolons at *c* in several cases may indicate a more or less unstable condition of polarity near the middle of the piece; but as a whole, the table shows a stronger tendency to produce hydranths at *c* (oral) than at *b* (aboral), *i. e.*, the original polarity in most cases overbalances whatever change may have been effected by the development of a hydranth at the aboral end of the original piece.

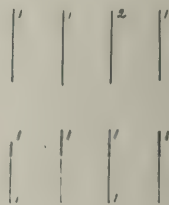


FIG. 4.



change in polarity had occurred. After three days eight of the pieces had polyps on the old oral ends and three had stolons on the old aboral ends (where the new polyp had developed); two pieces had heads on the old aboral end; one had a head on each end, and eight had not yet regenerated. After another day the record of these pieces was as follows: Twelve had polyps on the old oral end; seven had polyps on the old aboral end (six of these had oral heads also); five had stolons on the aboral end (of these four had a polyp on the old oral end and one had none). After another day fifteen had polyps on the old oral end; eight had polyps on the old aboral end; five had heads on both ends and four had long stolons on the old aboral end. These results show that despite the fact that the oral end was stuck into sand, the original polarity of that end is markedly shown when it is cut off. It is equally clear that the development of a polyp at the aboral end has changed the condition of that end so that it is more likely to produce a polyp (when it is cut off near the new aboral hydranth) than before. Thus the polarity of the whole piece is not reversed but a new growing region is established at the old aboral end. Another point in this connection is not without interest; namely, that the end in the sand begins to form an oral polyp in this species of *Tubularia*, and the process may go so far that the hydranth is fully formed and ready to come out. It fails, however, to emerge, unless removed from the sand, and is soon absorbed. Meanwhile the aboral hydranth has regenerated. We shall consider this point more fully later.

*Experiment 4.* In order to show more precisely the limits of the change that takes place at the aboral end as a result of the development there of a polyp, the long pieces were cut into many short ones. In the records given in Tables IV and V double-headed pieces were used; in the second series, Tables VI and VII, the oral ends of long pieces had been stuck into sand or vaseline and kept there until the aboral polyp appeared. As, however, in most cases, a hydranth forms at the oral end in sand or vaseline, such pieces are practically double-headed pieces. The pieces were then cut up into shorter ones varying from 5 mm. to 10 mm. or more in length. The development of the shortest pieces may



be somewhat retarded, that of the longest pieces little if at all. Since in each set the pieces were of approximately the same length, the retarding influence is probably constant for each

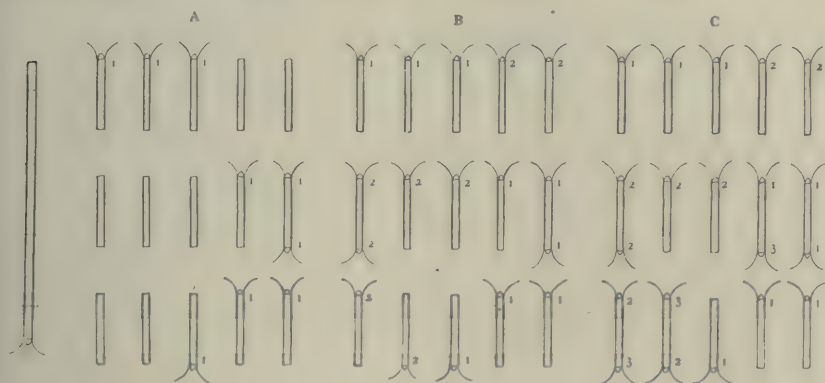


FIG. 5.

series. Fig. 5 gives an idea of the way in which these pieces behave.

		13		1	18		12		19	
	15			14		17	14			
14	12		1	1		1	14	1	14	
14	12	13	1	1	15	1	14	13	14	
		15		13	12	1	14		13	
14				12	12	14				
14				12		14				
				12	12					

TABLE IV.

The remaining series are given in a different form. In each case the old oral end is at the top in the series. Those polyps that emerged simultaneously have the same number; thus the first to emerge are numbered 1, the second 2, etc., the figures being placed near that end at which the polyp formed. In Tables IV and VI the figures 1, 2, 3, 4, 5 indicate observations made once a day for five successive days. In Tables V and VII the figures indicate the sequence for

1	1	1	12	12
1	12	12	1	1
12	1	1	1	1
1	12	1	1	1

TABLE V.

each long piece, observations having been made three or four times daily. A figure opposite the middle of a piece indicates a double structure that did not emerge.

<sup>12</sup> 			<sup>3</sup> 	<sup>3</sup> 	<sup>12</sup> 	<sup>3</sup> 	<sup>12</sup> 	<sup>3</sup> 	<sup>3</sup> 	<sup>12</sup> 	<sup>12</sup> 
<sup>12</sup> 			<sup>3</sup> 	<sup>4</sup> 	<sup>12</sup> 	<sup>12</sup> 	<sup>12</sup> 	<sup>3</sup> 	<sup>3</sup> 	<sup>12</sup> 	<sup>12</sup> 
<sup>3</sup> 	<sup>4</sup> 		<sup>3</sup> 	<sup>3</sup> 	<sup>12</sup> 	<sup>12</sup> 	<sup>3</sup> 	<sup>12</sup> 	<sup>3</sup> 	<sup>3</sup> 	<sup>12</sup> 
<sup>3</sup> 			<sup>3</sup> 	<sup>12</sup> 	<sup>12</sup> 	<sup>3</sup> 		<sup>3</sup> 	<sup>12</sup> 		
<sup>3</sup> 				<sup>12</sup> 	<sup>3</sup> 			<sup>3</sup> 			
				<sup>12</sup> 							
				<sup>3</sup> 							

TABLE VI.

instead. Whether the single case of a double-headed piece in this table (and by inference in other results when the piece is not so

In order to contrast the development of a series of short pieces cut from unaltered stems, *i. e.*, stems with only an oral polyp, one small series of such pieces was observed with the following results (Table VII):

Here the oral ends of the pieces produce polyps, and there are few aboral polyps. It is interesting to note in the third column that something has delayed the development of the oral ends of two pieces and aboral polyps have developed in-

<sup>3</sup> 	<sup>3</sup> 				<sup>12</sup> 	<sup>12</sup> 													
<sup>3</sup> 	<sup>12</sup> 				<sup>12</sup> 	<sup>12</sup> 			<sup>12</sup> 	<sup>12</sup> 			<sup>12</sup> 	<sup>12</sup> 			<sup>3</sup> 	<sup>12</sup> 	<sup>12</sup> 
<sup>12</sup> 	<sup>12</sup> 					<sup>12</sup> 	<sup>12</sup> 				<sup>12</sup> 	<sup>12</sup> 					<sup>12</sup> 	<sup>3</sup> 	<sup>12</sup> 
<sup>12</sup> 	<sup>12</sup> 				<sup>3</sup> 	<sup>12</sup> 				<sup>12</sup> 								<sup>3</sup> 	<sup>12</sup> 
					<sup>12</sup> 														

TABLE VII.

short that the tentacle areas of the ends overlap) can also be accounted for in this way, can not be established from the evidence, although it seems not improbable.

*Experiment 5.* The rate at which the hydranth forms is, it appears, an important factor in connection with the phenomenon of heteromorphosis. A series of experiments was undertaken to determine whether the amount of material in the circulation has an influence on the rate at which the hydranths appear. This and the five following experiments have a bearing on this point.

In several series a long piece was cut out and its ends allowed to close. Then at intervals small pieces were cut from the aboral end. Each time that a small end-piece is removed a part of the circulating fluid with its contained granules escapes from the long piece into the water. The cut end closes in the course of an hour making further escape of fluid impossible. It is true that a small amount of new material is added from the ridges at the aboral end each time the piece is cut, but it appears that not enough is added to compensate for that which is lost. The greatest amount of pigment, etc., always comes from the endoderm of the oral end where the changes preparatory to the formation of a hydranth takes place. The following are the records of the results. A check series of pieces, not cut at intervals at the aboral end, was kept in each experiment. In one set the long pieces were cut at 10.00 a. m., July 15, and at 3.00 p. m. the aboral ends were cut off. The next morning (July 16, 9.45 a. m.), 18 of these pieces showed the primordia of tentacles, two had none. The check pieces were in the same condition. At 4.10 p. m. none of the polyps was out. The next morning (July 17, 9.00 a. m.) 11 hydranths were out, 6 showed the primordia of polyps, one nothing. Of the check series, 9 polyps were out, and 3 pieces showed primordia. The following morning 16 polyps were out, and 2 not yet out; of the check set 10 were out and 3 not yet out.

In another set (July 31) the pieces were cut off at 11.30 a. m. The first aboral cut was made at 5.50 p. m., and the second the next morning at 9.00 a. m. At this time the beginning of the

'	'	l	°	
°	'	°	°	°
'	°	l	°	°
'	°	°	°	°
			°	°

TABLE VIII.



polyp could be faintly seen. At 10.00 a. m. on the following day (August 2), 6 polyps were out, 1 was coming out and 1 showed nothing. Of the check set, 4 were out and 1 had the primordia present.

In a third set the pieces were cut out at 10.45 a. m., July 30. The first aboral cut was made at 2.35 p. m., the second at 6.00 p. m., and the third the next morning (July 31) at 9.40 when the primordia were present. After another 24 hours (August 1, 9.45 a. m.) the polyps were all out in the normal time. These results show that by opening the stem and allowing the escape of fluid, the development of the polyp is not retarded.

*Experiment 6.* In two experiments short pieces were cut from the oral end at the same intervals as in the last series; Experiment 5. This operation suffices to let some of the fluid and the collection of pigment escape. The polyps were slightly retarded (emerging at 3.00 p. m., August 1), which is not surprising when it is recalled that the cuts removed a small portion of the distal end of the hydranth.

In another series the pieces were cut off July 14, at 2.30 p. m., the first oral cut made at 6.00 p. m., and the next day (July 15) two cuts were made at 10.00 a. m. and 3.00 p. m., respectively. The following morning (July 16, 9.30 a. m.) another cut was made when the primordium of a polyp was present on 4 pieces. The check pieces were in the same condition. The polyps did not emerge until July 17, when proportionately as many were out as in the check series. There was a slight delay caused by the cutting, owing no doubt to too much of the polyp being removed, but since the primordia formed at the same time as in the check series, it is clear that the development up to this stage was not delayed by removing very short pieces of the oral end of the stem at four different times. Very small pieces only should, of course, be removed, for if a longer piece is removed, the region of tentacle formation is cut through, which will cause a delay in subsequent development. The most distal part of the hydranth-forming region goes to make the proboscis and may be removed in the early stages without interfering seriously with the development of the hydranth.

*Experiment 7.* With a fine needle it is possible to puncture the membrane over the closed aboral end of the piece and thus to allow a partial escape of the fluid. Two series of experiments of this sort showed that the oral polyp formed as soon as in the check pieces.

*Experiment 8.* By tying a succession of ligatures of silk thread around the aboral end it was hoped that more material might be thrown into the circulation; and if its presence had any influence on the rate of polyp-formation, the hydranths should appear sooner than in the check pieces. It was found, however, that no appreciable addition of material could be made in this way, since so little of the ridges breaks down at the aboral end. There was no hastening of the development after tying two or three times. The details may be omitted.

*Experiment 9.* A more successful method of introducing material into the current consists in rolling a glass rod from behind forward for a short distance over the aboral end. In this way, as Godlewski has shown, the cœnosarc may be rolled into the interior of the more anterior part, and we have found that some at least of the plug may at times enter the circulation. Two series of experiments showed that the oral hydranth cannot be hastened in this way.

*Experiment 10.* By crushing the aboral end it is possible in some cases to introduce a large amount of substance, including the red pigment, into the circulation. Comparing the rate of development of pieces containing a great deal of material in the circulation, with other pieces containing very little, no difference in time of polyp-formation was detected.

*Experiment 11.* This experiment was undertaken in the hope of determining what changes take place in the piece during the formation of the oral hydranth. Long pieces were cut out, and then at different intervals (8, 12, 18, 24 hours, etc.) they were cut up into short pieces (5-6 mm.), which were placed in a row and the time when their polyps appeared recorded. If there is a movement forward of material in the wall, or if material in the wall is slowly used up from the oral to the aboral end, we hoped that this might be demonstrated by the time of development

			3					
		2		2	4	2	2	2
		3	2	4	3	3	3	
		2	2	3	2	4	2	
	2	3		3	2		2	

TABLE IX (6 hours).

	3				3
5	3	3	5	2	
	x		x		x
4		2	5	4	
			x		x
3	3	2	2	4	
			x		
2	3	2	4	3	
			x		
3			3	3	
			x		
2					2
					x

TABLE X (15 hours).

4g)					
	2	2	2	2	2
x					
2	3	3	3	3	3
2	4	5	3	3	3
3	3	4	4	4	4
3	3	3	5	4	2
4	3	3			
x					
			4		

TABLE XI (16 hours).

4g)					
	2	2	2	2	2
x					
2	3	3	3	3	3
2	4	5	3	3	3
3	3	4	4	4	4
3	3	3	5	4	2
4	3	3			
x					
			4		

TABLE XII (20 hours).

			4g)		
2	2	2		2	
3	3	3	2	3	2
4	4	3	3	4	2
3	3	3	3	3	
6			3		
x					

TABLE XIII (24 hours).



in the series of short pieces. So much irregularity occurs, however, that it is doubtful if the result establishes any definite conclusion.

In the first series the long pieces were cut into short pieces (5-6 mm.) after 6, 15, 16, 20, 24 hours. This series, kept in a warmer room, regenerated much faster than the second series. The figures indicate the sequence of the oral polyps, and  $\times$  indicates an aboral hydranth.

After 6 hours, as shown in these tables, the intervals between the time of emergence of the polyps in succeeding pieces are not very different, although, as a rule, the piece nearest the oral end developed first. After 15 hours the irregularity is so great that no conclusion can be safely drawn. The following series, after 16 hours, shows a slightly regular sequence from oral to aboral end. After 20 hours a greater regularity is observable, and it will be recalled that at about this time the primordium of the polyp can usually be seen. Also after 24 hours the sequence is more marked.

In the next series the intervals were different, the short pieces averaged less in length and the development was very much retarded, largely due to lower temperature, as compared with the preceding series. The figures indicate observations 24 hours apart.

In the check series the pieces from the same stem developed in about the same time, although in some cases the more basal pieces were later. In the third row the most aboral piece produced a stolon and later its end developed a polyp. The 8-hour series is not good, but so far as it goes, it shows no more than the check series. The 12-hour series is not different from the preceding. The 18-hour series shows no special influence of the changes that were going on at the oral end of the piece before it was cut into small pieces. The first two rows developed very slowly. The 24-hour series developed much better, *i. e.*, earlier than the preceding, but while in most cases the oral pieces developed first, there was not a relatively great difference between these and the more aboral pieces. After 36 hours there is a decided difference shown by the oral pieces, the remainder of each set being much the same, and this is also true for the 48-hour series.

12	12	12	13
12	13	12	13
1	1	1	13
1	1	15	14
1	1	17	17
1	1	1	14

TABLE XIV  
(8 hours).

[illegible]

TABLE XVII (24 hours).

		<sup>2</sup>	<sup>3</sup>			<sup>2</sup>	
	<sup>2</sup>	<sup>3</sup>				<sup>2</sup>	
	<sup>2</sup>	<sup>3</sup>				<sup>2</sup>	
<sup>c</sup>		<sup>3</sup>				<sup>2</sup>	
		<sup>3</sup>				<sup>3</sup>	<sup>2</sup>
	<sup>v</sup>					<sup>3</sup>	<sup>3</sup>
<sup>3</sup>		<sup>4</sup>	<sup>3</sup>		<sup>3</sup>	<sup>3</sup>	
	<sup>r</sup>						
							<sup>3</sup>

TABLE XV (12 hours).

1' 1' 1' 1' 1'  
 13 14 15 13 16  
 13 13 13 14 17  
 13 14 13 15 15  
 13 15 13 16  
 13 15 13 14

TABLE XVIII (36 hours).

1	3	1	1	1	1	1
1	3	1	5	3	3	1
1	3	1	6	4	4	4
1	1	1	1	3	4	4
1			1			

TABLE XIX (48 hours).

7	8	3	2	2	3
6	6	3	2	2	2
5	8				
	4	3	2	3	3
5					
6		3	3		2
6		3	3	6	
6			3	3	

TABLE XVI  
(18 hours).

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25
26	27	28	29	30
31	32	33	34	35
36	37	38	39	40
41	42	43	44	45
46	47	48	49	50
51	52	53	54	55
56	57	58	59	60
61	62	63	64	65
66	67	68	69	70
71	72	73	74	75
76	77	78	79	80
81	82	83	84	85
86	87	88	89	90
91	92	93	94	95
96	97	98	99	100

TABLE XX (check).

The second series developed slowly as has been said, owing to colder weather. Even the long pieces, kept 48 hours before cutting, had not pushed out their polyps, although the primordia had begun to appear. In the 24-hour series, the primordium was so far developed that it could be easily seen, and after 36 and 48 hours the hydranths were fully formed and ready to emerge. Whether we can correlate with this latter condition the relatively more rapid development of the pieces that lay just behind the oral region is perhaps questionable, but it appears not improbable.

The main result of the experiment is negative; for, no satisfactory evidence was obtained to show what changes are taking place in the stem during the formation of the oral hydranth, except perhaps in the case of the pieces immediately behind the hydranth-forming region in the series cut after that region was well developed. It will be noted that there are fewer double-headed short pieces in these sets than in those cut from double-headed long pieces. Since the shortness of the pieces may have caused a delay in development and thus introduced another factor, we tried the following experiment in which long pieces were used.

*Experiment 12.* Here long pieces were cut from the stem, and after certain intervals, the oral end was cut off at two or three different levels, and the time required for the development of a polyp was noted and compared with that in a check series in which the old hydranth was cut off at the time of the second operation on the long pieces. In one set the long pieces were kept 12 hours after the old head had been cut off, and then about 4 mm. of the oral end was cut off in some pieces, in others about 15 mm., and in others a much longer piece. Check pieces were cut at the first and last of these levels at the same time. No difference in the time of regeneration at the three levels or in the check experiment could be detected.

Other pieces of the same set were cut off after 17 hours and gave the same result. In another set the long pieces were kept 24 and 36 hours (when the primordia of the polyps had appeared) and the oral ends were then cut off at three levels, (*a*) just behind the primordium, (*b*) 10 to 20 mm. further back, and (*c*) far back nearer the base of the stem. Check series were also made. Here



again no difference could be detected in the rate of regeneration at the three levels or in comparison with the check series. These results seem to indicate that the rate of development is not determined by the nearness of the new cut surface to the region where a hydranth has developed, and thus supports the view that the material that is made use of in the formation of a polyp comes mainly from the polyp-forming region and not from the whole stem (except perhaps to a slight extent from the broken down ridges from the aboral end as will be spoken of below.) It is true that pieces kept under artificial conditions regenerate after a time less rapidly than fresh pieces, but this may be due to general exhaustion of food substances throughout the piece, so that there is relatively less material from which to produce a new polyp.

*Experiment 13.* The more rapid development of the aboral polyp when a ligature is tied around the stem has already been noted by Driesch, Morgan and Loeb. In *Tubularia marina* the aboral polyp so seldom develops in pieces left on the bottom of a dish that this method and that of sticking the oral end into sand or vaseline is the best way to obtain heteromorphic regeneration. Loeb found that it makes little if any difference in the time of appearance of the hydranth, how far the ligature was from the basal end. As this result has a direct bearing on our problem we repeated the experiment a number of times and under somewhat different conditions. In the first series some pieces (cut very long) were tied August 1, 10.00 a. m., near the polyp, others near the middle of the stem, and others near the basal end. Three days later one tied in the middle had an aboral polyp; seven hours later one of the pieces tied near the aboral end also had a polyp. During the four following days no other aboral polyps developed. In another series (August 1, 10.15 a. m.) after three days, one piece tied near the polyp, one tied in the middle, and one tied near the aboral end had polyps. The next day another tied near the polyp had regenerated, and the day following another tied near the polyp had an aboral hydranth. No more developed during the next two days.

In another series (August 1, 5.00 p. m.) one piece tied near the

aboral end developed in two and a half days, and during the four following days no others developed.

In another set, after three days, one piece tied near the polyp and one tied near the basal end had regenerated, and three days later one of those tied near the middle developed a hydranth.

In another set, after four days, one tied near the polyp and one tied near the middle regenerated, and after three days more one tied near the basal end developed.

In two sets no aboral polyps developed.

These results, as well as other similar ones, confirm Loeb's statement that no definite relation exists between the time of development of the aboral polyp and the length of the piece of stem between the ligature and the basal end. Individual stems behave very differently, but no connection between rate and length of aboral pieces is apparent. So great is the difference in time in different cases that difference in rate of development due to another factor might be difficult to detect without more data. In this connection the regeneration of the oral polyp, which takes place more rapidly should be considered. Long pieces regenerate a little sooner than pieces 7-12 mm. long, and in shorter pieces (5 mm. or less) there is a longer delay. If a similar influence was present at the aboral end in this experiment it might not be detected. However, the length of the stem does not appear to be, in either case, the principal factor that determines the *rate* of aboral regeneration.

*Experiment 14.* Another series of experiments, similar to the above, was carried out in order to determine what influence the changes involved in the development of an oral polyp have on the rate of development of an aboral polyp.

(a) A check series of 10 pieces 15 mm. long was cut and tied in the middle as soon as cut. The oral hydranths (10) came out in from 19 to 68 hours, average 49.9 hours. The aboral polyps (9) appeared in from 46 to 76.5 hours, average 69.8 hours.

(b) 10 pieces 15-20 mm. long were tied in the middle 8 hours after they were cut. The aboral hydranths (8) came out on an average of 77.8 hours, just 8 hours later than those that were tied at once.

(c) 9 pieces 15–20 mm. long were tied 16 hours after they were cut. The aboral polyps (8) appeared on an average of 83.3 hours, 15.5 hours later than those tied at once.

(d) 19 pieces 15–20 mm. long were tied 24 hours after they were cut, 7 in the middle, 5 about 5 mm. from the oral end of the piece and 7 about 5 mm. from the aboral end.

The average time for the aboral polyps (7) in the pieces tied in the middle was 94.1 hours—24.3 hours more than for those tied at once. For those tied near the oral end (4) the average was 101.7 hours, and for those tied near the aboral end 102 hours—31.9 and 32.2 hours, respectively, more than for pieces tied at once.

From the results obtained in all but the last two sets of pieces, it appeared evident that it is immaterial whether the pieces are tied when cut, or from 8 to 24 hours later, *i. e.*, the changes that were going on at the oral end before the pieces were tied had no retarding effect on the aboral hydranths.

The discrepancy observed in the case of the pieces tied near the oral and aboral ends after 24 hours, was so great that the experiment was repeated twice. The results, however, were too erratic to be reliable. The peculiarities were probably due to individual differences in the stems used, as other conditions were apparently the same. Several aboral stolons were produced in both of these latter sets.

In connection with this experiment Loeb's experiment of cutting out pieces between the ligature and the aboral hydranth was repeated. In one case the oral and aboral hydranths appeared at the same time; two pieces, cut very near the aboral polyp, developed aboral hydranths and nothing at the oral end; six pieces had oral polyps and stolons or nothing at the aboral end. The evidence, though not abundant, is in favor of the supposition that the polarity of the whole piece is but slightly affected by the development of an aboral polyp.

*Experiment 15.* Several long pieces, which had regenerated a hydranth at the oral end and a stolon at the aboral end, had their oral ends stuck into sand without cutting off the hydranth, and after 4, 5 or more days a hydranth was formed at the uncut



free end of the stolon. These hydranths were formed in the usual way and developed as rapidly as oral polyps after the primordia appeared, but a longer time elapsed before they began to form than in the case of aboral cut ends of pieces stuck into sand. All the pieces of this kind (14) produced hydranths and in several cases the hydranth degenerated and regenerated several times in the course of a month. The same result was obtained when uncut pieces bearing stolons were tied. When the stolons were cut off, a hydranth was formed very quickly at the cut end (oral) of young (recently grown) stolons; pieces of old stolons usually regenerated a hydranth at the oral end and a stolon at the aboral end.

*Experiment 16.* The above experiment suggested the following: Several long uncut stems were ligatured at two or three places to see whether hydranths would develop between the ligatures, *i. e.*, in parts of the cœnosarc cut off from the rest of the piece, but with the ends not exposed to sea water.

In the first set 8 stems were used and one oral hydranth developed between the ligatures after 6 days. In the second 7 stems were ligatured. One oral hydranth appeared after 7 days, one aboral after 13 days, and another oral after 20 days. The first set might have yielded further results if they had been observed for a longer time. Experiments 15 and 16 show that a hydranth may be formed at an end of stem or stolon not exposed to sea water by cutting the perisarc, but the development of such hydranths is long delayed. The absence of a hydranth, however removed, whether by natural degeneration, cutting, burying in sand or ligaturing, seems to lead to the regeneration of another hydranth, but the rate of regeneration depends on other conditions.

*Experiment 17.* In connection with Experiment 2, it occurred to us rather late, when little available material was at hand, that long double-headed pieces should be cut in the middle, without removing the hydranths, to see whether the two cut ends would behave alike or differently. Only one piece regenerated and that produced stolons on both cut surfaces, regeneration being most rapid at the aboral end of the distal half of the piece.

*Experiment 18.* Delayed development in several series of short pieces suggested the possibility that in this species light might be a factor in the rate of regeneration. To test this three sets of similar pieces were placed (*a*) in bright light near a window, (*b*) in diffuse light, (*c*) in total darkness. The rate of regeneration for the three sets was the same.

*Experiment 19.* The polarity of stolons was examined by means of the following experiments: Young stolons that had developed during four or five days and were stuck to the dish were cut off, and each one produced a hydranth at the cut end. Old stolons by which the *Tubularia* had been attached to rocks were also cut into pieces 5–10 mm. long and kept oriented. In 10 cases the polyp appeared on the end nearer the free end of the stolon; in 18 cases on the end nearer the stem. Pieces cut from recently grown stolons which had produced a polyp at the free end, regenerated a hydranth at the end nearer the polyp. These results show that the “polarity” of the stolon is not so marked as that of the stem. When it is recalled that the free end of the stolon may produce a hydranth if it does not come in contact with a hard surface, the behavior of the two ends of the pieces is not so remarkable. The free end of a stolon behaves in most respects like an aboral, exposed end of a stem which also, under certain conditions, produces a polyp.

*Experiment 20.* In order to see to what extent changes preparatory to the formation of a polyp have taken place at the aboral end of a long piece that has regenerated an oral hydranth, pieces that had within two or three days produced an oral polyp were used. The tip of the aboral end was cut off (in most cases) and the piece removed near the middle or nearer the aboral end. If changes preparatory to the formation of a polyp had taken place, we should expect the aboral polyp to develop first unless the original polar conditions enabled the oral end to outstrip the aboral end despite the advantage the latter might be supposed to have gained by exposure to sea water. The results showed that in 29 cases the oral polyp only developed, in 6 cases the aboral, in 1 case oral and aboral at the same time, and in 1 case the aboral  $6\frac{1}{2}$  hours before the oral. A higher percentage of aboral polyps developed

than when pieces are cut out of stems that have not previously been cut, and this may be due to the changes already begun at the aboral end; but on the other hand the large predominance of cases of regeneration at the oral end indicates that the changes at the aboral end are slight, and that the original polarity of the material is still the more important factor.





# AN ATTEMPT TO ANALYZE THE PHENOMENA OF POLARITY IN TUBULARIA.

BY

T. H. MORGAN.

Two questions arise in connection with the problem of polarity in *Tubularia*: (1) The *kind of structure*, polyp or stolon, that develops on a cut surface; and (2) the time at which the new structure appears. The former includes the idea of "polarity" in the usual sense; but, as I shall attempt to show, the time of appearance of the new structure may also be an important factor in determining the kind of structure that develops when an alternative exists.

A cut surface at any level may produce a polyp or a stolon. Usually polyps appear on distal cut surfaces, stolons on basal ones. An external stimulus, viz: exposure of a cut end to water, calls forth the development of a hydranth and the hydranth may develop either on a distal or on a basal surface. If a piece is open at both ends, the oral hydranth develops first, and after it has emerged, the aboral end may also produce a polyp, rather infrequently in *Tubularia marina*, but more often in *T. mesembryanthemum* and *T. crocea*. On the other hand, if the stem is tied in the middle, the basal polyp develops sooner than when the stem is not tied, and it appears in practically every instance. The aboral development takes place to a large extent independently of the length of the stem between the ligature and the aboral end.

How can we account for these facts? We can at least formulate a provisional hypothesis. We may assume that the gradation of the material is of such a kind that the hydranth-forming material decreases from the apical toward the basal end. The formative influence, acting from the exposed end inward (the stimulus of the water on the free end), finds a prompter response when it acts in the direction of decreasing amounts of hydranth-forming

material (which has the same gradation as that in the hydranth itself) than when acting in the reverse direction (namely, at the aboral end). Therefore, the oral polyp, as a rule, develops first. For its development it needs certain nutritive material. This it finds either in the *cœnosarc* or in the circulation, and uses the material as it develops. In consequence the cut surface at the basal end cannot get the material necessary for it to develop into a hydranth, and it either remains undeveloped or produces a stolon. If, however, we tie a ligature around the piece, the aboral end may then make use of the substances that are present in the isolated aboral part of the piece; hence there develops a hydranth. Its development is usually delayed as compared with that at the oral end of a piece cut off at the same level. Why does this delay occur? It can be accounted for in either of the following ways. It may be assumed that even when the stem is tied the oral end near the ligature often begins to form a polyp, and this developing end uses up at first all of the available food material, but as development at this end stops the stimulus of the water on the aboral end makes that end active in the presence of an excess of material. That the oral polyp may actually begin to develop or even completely develop when the stem is tied, or when one end is stuck into sand, or into vaseline, has been shown by our experiments. On the other hand it is important to note that the assumed gradation in the material from hydranth to base must be first reversed before the aboral hydranth can develop. The necessity for reversing the arrangement may in itself account for the delay in the development of the aboral hydranth, and for the prompter development of the oral one.

As has been said, the hypothesis that I advocate is, in principle, fundamentally different from that of the Bonnet-Sach's view, more recently advocated by Goebel and by Loeb. I assume that the results are not due to the movement in a definite direction of a formative or even of a nutritive material that causes the development of a hydranth. On the contrary, the hydranth begins to develop and it then makes use of the material that comes to it. If in special cases, such for instance as those where no circulation is present, the using up of material causes a movement of nutritive



substances toward the place where they are consumed, such a movement is the effect of the development and not its cause.

In the case of *Tubularia* it is clear from many experiments that the rotary circulation in the stem (which is the result of ciliary action) can not be made to account for the polarity of the stem. The red pigment does not appear to be a nutritive or a formative material. It seems to bear no other relation to the regeneration than that of a waste product. The remaining material of the ridges that is set free along with the pigment may be, and probably is, a nutritive substance that is made use of by the developing hydranth.

Some other possibilities involved in my hypothesis may be briefly mentioned. Experiments have shown that when a polyp develops on the aboral end some change takes place, especially in the region immediately behind the polyp, of such a sort that we may say the "polarity" of that region has been changed. This means, on my view, that by the development of a polyp on this end the region behind the polyp has been also changed and is more like the region behind a normal polyp. Consequently a *short* piece in this region may produce an aboral polyp (in the original sense) before an oral one. How far this influence extends is not conclusively shown, but the effect is certainly stronger just behind the new aboral polyp, and it probably diminishes rapidly in the original oral direction.

A possible objection to my view may be made on the following grounds: If pieces of medium length contain enough food material to produce oral polyps then, if food stuff were made throughout the piece, there should be an excessive amount in long pieces, and aboral as well as oral polyps should develop in the latter, which is not the case. The answer to this objection is that the change in the food stuffs that makes them soluble probably takes place only, or largely, in the region where the polyp is developing and not throughout the piece. The soluble material set free at the aboral end as well as at the oral end is utilized at the oral end, because that end develops first. The actual observations suggest, if they do not prove, that the available material may be added to by the protoplasm and its food contents set free from the endodermal ridges.

It was attempted in one experiment to determine how far the preliminary changes that take place in the aboral end represent steps leading toward the development of a hydranth at that end. A very short piece was removed from the aboral end of a long piece (to make a new surface there) at the time when the oral end had just made a polyp. The long piece was then also cut in two in the middle. It was hoped in this way to find out if preliminary changes, leading toward the development of an aboral polyp, which had been going on at the aboral end, would lead to the aboral polyp developing first. It was found that usually the oral polyp developed first, indicating that little if any change had taken place at the aboral end in the direction of polyp formation. From this result it is not improbable that the slight breaking down of the ridges at this end may not be connected with polyp formation there, but possibly only with the closure of that end. Occasionally, however, the aboral end has changed so that it produces its polyp first—a result that never occurs in this species when long pieces of the normal stem are cut off.

The result of experiments with stolons is in some respects more difficult to explain, but may still be brought into line with the remaining results. When the stolon is cut off it generally produces a hydranth on that end that was nearest to the original polyp. In other words, it behaves like a piece of the stem. In quite a large number of cases, however, the hydranth appears at the apical end. This is not in harmony with my view, unless we assume that the differences in the two ends of the stolon, *especially of a very new one*, are such that local conditions (the action of water on the new end) may be stronger at times than the differences in the two ends. Moreover, it is not to be forgotten that a stolon, not in contact with a surface, tends to produce a hydranth on its *closed* end; especially if the oral hydranth is suppressed or the oral end of the stem closed. The newness of the growing tip may also make it more responsive to the action of water if it is not in contact with a surface.

A postulate of my view is that the polyp after it has formed continues to use up nutritive material as long as it grows, and hence tends to hold in check for some time after its first formation

the development of a polyp at the aboral end. The polyp when first formed is small, and can, in fact, be seen to enlarge for several days after it has emerged.

I have assumed that the stem of *Tubularia* is not homogeneous, but that from the hydranth to the base there is a graded difference and this gives the order or stratification of the material. The stimulus of the water acting on the free end arouses the formative changes which act in the direction of this existing material order. How does such a view differ from the old assumption of a "polarity" in the material? On my view there is no such directive force residing in the material as the term polarity suggests, but the polarity is only a name for the gradation of the material and on this as a basis the formative changes are carried out.

Conversely a similar gradation from the stolon to the hydranth must exist in respect to the tendency to produce a stolon, hence the latter appears on the aboral end; but in the case of *Tubularia* we find that the action of water on a free end, whichever this may be, has a stronger tendency to call forth a hydranth than a stolon in some species. Hence the conflict of influences that probably goes on at this end. If, however, the aboral end is brought in contact with a solid body a stolon develops promptly, while the oral end, if brought into contact with a solid, fails to produce a stolon, but develops a hydranth.

In conclusion, I think, that the Sachs-Bonnet hypothesis of the *migration* of formative stuffs is not needed to explain the results in *Tubularia*, and furthermore that there are no observed facts or experiments that make this view probable. I have no wish to deny that substances having a formative influence on growth may exist, but there is no evidence in favor of the view that *in multicellular forms*, such substances migrate in definite directions and thus produce the "polarity." If development begins in a region, there may be a movement of the materials that are being used up toward the developing part, but to fail to distinguish between this point of view and the former is to confuse cause and effect. The two points of view are diametrically opposite, and no good end can be reached by ignoring this fact.





# REGENERATION IN LARVAL LEGS OF SILKWORMS.

BY

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WITH 10 FIGURES.

As far as mentioned by Morgan<sup>1</sup> and Brindley,<sup>2</sup> which are the only two recent accounts, known to me, that attempt to refer in an inclusive way to the recorded observations and experiments on regeneration in insects, all<sup>3</sup> the work done on regeneration of the legs in insects with complete metamorphosis has been limited to making mutilations of the larval legs and noting what, if any, effect was apparent in the legs of the imago. There are several accounts of such observations, and some of these accounts are, curiously enough, of comparatively recent date. I say "curiously," for it has been known now for a score of years and more, that the legs (as also the wings, antennæ, etc.) of insects of complete metamorphosis are derived (at least in all the higher forms, such as the *Lepidoptera*, *Diptera* and *Hymenoptera*) not by a transforming of the larval legs (if present) into the imaginal ones, but from new centers called imaginal discs or histoblasts. These histoblasts are developed from an invagination of the larval cellular skin layer (hypoderm) and only in comparatively late larval life do the new developing imaginal legs lie within the larval ones. It follows from this that if a larval leg be cut off in early larval life the imaginal leg is in no way mutilated, and that if it

<sup>1</sup>Regeneration, 1901.

<sup>2</sup>On Certain Characters of Reproduced Appendages in *Arthropoda*, particularly in the *Blattidæ*. Proc. Zool. Soc., Lond., 1898, pp. 924-958.

<sup>3</sup>Tornier describes (Zool. Anzeiger, Vol. XXIV, pp. 634-664) certain experiments on regeneration in the meal beetle, *Tenebrio molitor*, in which account he states that cut-off larval legs are regenerated before pupation, if young larvæ are used as subjects. This statement of Tornier's I have only found since sending my paper to press.

appears of full size and normal character in the adult insect, this is not due to restorative regeneration, but simply to its normal growth and development. If a leg be cut off in late larval life, the developing imaginal leg may or may not be at the same time mutilated. If mutilated, however, it will always be by a removal of much less of its extent than of the extent of the larval leg taken off. A cut which severs the larval leg near its base (for example, through the base of the femur,) will not take off more than the tarsus or perhaps part of the tibia and tarsus of the imaginal leg, which, in its development, is beginning to extend into the larval one. Thus if the imaginal leg be found, when the imago issues, to lack a tarsus but to possess a complete femur and tibia, this is no indication that there has been a partial regeneration; there may have been none whatever.

To make a definitive test of the capacity of an insect with complete metamorphosis to regenerate lost parts I have cut off legs, both thoracic and abdominal (prop-legs) of the larvæ of the silkworm moth, *Bombyx mori*, at various ages, and have noticed whether or not regeneration of these legs took place before pupation, and if so in what degree and whether normally, *i. e.*, so as to produce an exact replica of the lost leg, or not. The life of the silkworm larva is about 50 days (in the races which I have used for study, and under the conditions attending their rearing in my laboratory), and is divided by four moultings into five approximately equal, active, feeding periods. In the first and second period the larvæ are too small to operate upon satisfactorily, but after the second moulting the legs can be taken off at any particular level desired. In the experiments silkworms of several races, viz: Japanese white, Chinese white, Italian yellow, Chinese crossed, etc., were used but the regenerative phenomena in all were alike.

### *Regeneration of Legs.*

The results of the experiments may be stated and illustrated (see Figs. 1 to 10), as follows: In the first lots of individuals, mostly of six each, one thoracic leg or one abdominal (prop-) leg or one of each group of legs was cut off of larvæ about 15



day sold; that is, between the first and second moulting. Colloidization of the wounds was first tried, as it was believed that with such a turgid body and with the kind of circulation possessed by the silkworm the loss of blood-lymph would be considerable. It was soon noted that the blood loss was small and the wound

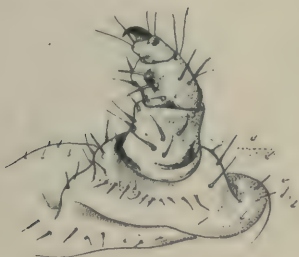


FIG. 1.



FIG. 2.

Fig. 1. Normal leg of third thoracic segment of full-grown larva.

Fig. 2. Normal abdominal prop-leg of full-grown larva.

quickly closed in nearly all cases. Most of the worms, roughly about 80 per cent, lived, and went through their subsequent moultings normally. After the second moulting, which was the first moulting after the loss of the legs, the wounds were always cleanly covered over by new skin, and no sign of regeneration nor of scar was apparent. After the next moulting, however, some specimens would show a certain obvious degree of regeneration, both thoracic and prop-legs being replaced more or less nearly completely as regards number of segments, size, and character of the distal tip. Some specimens would however show no regeneration at all. Nor would these non-regenerating individuals show any change after the later (last) moulting. This was also apparently true of the regenerating individuals also; that is, the amount or character of regeneration shown after the second moulting after mutilation was not increased or changed in the later life of the larva, which has regularly another moulting before the time of spinning up and pupation.

The unevenness of the results in these cases, both in degree of regeneration and in the regular occurrence of a few cases of no

regeneration at all led me to modify the later experiments as follows: In one lot of worms one prop-leg and one thoracic leg were cut off in the case of each individual halfway between base and tip, or at least always somewhat above the base, while in another lot of larvæ taken at the same time, at the same age,

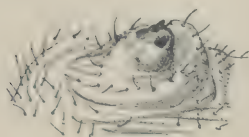


FIG. 3.



FIG. 4.

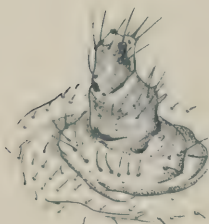


FIG. 5.



FIG. 6.

Fig. 3. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

Fig. 4. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

Fig. 5. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

Fig. 6. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

the legs were cut off as close to the body as possible. The results indicated that a condition, which I expected would be revealed, actually does exist. The larvæ with legs cut so as to leave a stump in all cases regenerated the leg more or less nearly completely, although in practically all cases of smaller size than the original; while those larvæ whose legs had been cut off as near the body as possible, *i. e.*, wholly removed, in no case regenerated a leg or any part of one. That is, the silkworm's leg can regener-

ate any part of itself, but the silkworm's body (trunk) cannot regenerate a leg wholly lost.

The structural characteristics of the normal thoracic legs and normal prop-legs, are shown in Figs. 1 and 2, respectively, while Figs. 3 to 8 illustrate cases of regeneration selected to show various

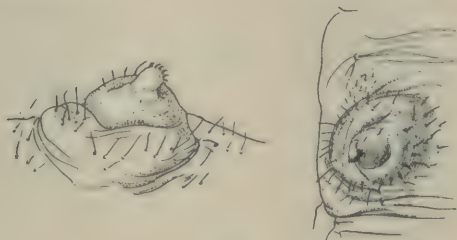


FIG. 7.



FIG. 8.

Fig. 7. Regenerated abdominal (prop-) leg of full-grown larva from stump of leg cut off just above base after first moulting; at left profile view; at right, ventral view.

Fig. 8. Regenerated abdominal (prop-) leg of full-grown larva from stump of leg cut off just above base after first moulting; at left, profile view; at right, ventral view.

degrees of it. Figs. 9 and 10 illustrate examples of no regeneration. In no case of regenerated leg was there a complete reproduction of the original in all details, but in all cases the evident tendency is plainly toward a replica of the original. In the case of the segmented legs (the thoracic) the original number of segments was usually reached and a small terminal claw was produced although always in reduced condition. In the case of the unsegmented prop-legs the terminal half circlet of hooks characteristic of the normal leg was in no case of regeneration completely reproduced, but in all cases a few at least of these terminal hooklets reappeared.



To sum up the results of the experiments, we may say, (a) that the larva of the silkworm moth, *Bombyx mori*, has the capacity of regenerating its thoracic and abdominal (prop-) legs from stumps of these legs, but not from the body (trunk), *i. e.*, that each leg has the capacity to regenerate any distal part from

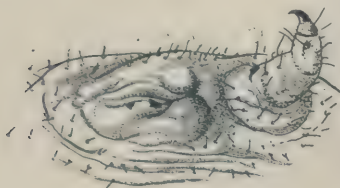


FIG. 9.



FIG. 10.

Fig. 9. Ventral aspect of third thoracic segment of full-grown larva, which had the left leg cut off at base after the second moulting; no regeneration.

Fig. 10. Ventral aspect of abdominal segment of full-grown larva, which had the left leg cut off at base after the first moulting; no regeneration.

any proximal part, but that the body cannot produce a wholly new leg; (b) that this regeneration shows externally not after the first moulting after the mutilation but after the second moulting, and that the regenerative processes are completed with the appearance of the new parts after this second moulting succeeding the mutilation.

### *Regeneration of Caudal Horn.*

The small caudal horn, a pointed non-segmented, but movable, process projecting upward from the dorsal surface of the penultimate abdominal segment was cut off in many larvæ (silkworms) of various ages, and in no case was there the slightest regeneration. After the first moulting succeeding the mutilation the new skin always extended smoothly over the place where the horn had been, without any sign of scar.

The function of this horn, which occurs on some other lepidopterous larvæ, notable and characteristically on the larvæ of the Sphingid moths, is unknown. It has been explained by some entomologists as an ornament, by others as a "terrifying

organ." It is not a sting nor in any way an effective weapon of defense, as even where long and conspicuous ( $\frac{1}{3}$  in. long) it is weak and easily bent. Nor does it secrete an acrid or ill-smelling fluid. Certainly in the silkworm it has had for many hundreds of generations no possible function as a weapon. It is interesting to note that this useless organ is not regenerated.

*Relation of Regeneration to Natural Selection.*

This suggests to us a consideration of the relation of regeneration, as we have observed it in the silkworm, to its causes, or at least to natural selection as an explaining cause. If the caudal horn is now a useless organ in the silkworm body its lack of capacity to regenerate (loss of capacity, if it ever had it) would seem to favor the theory of the natural selectionists concerning regeneration. At first glance, also, the retaining of the regenerative capacity of the legs, useful organs, may seem to favor this theory. But it must be borne in mind that the silkworm has been for approximately 5000 years a domesticated animal cared for under such conditions as to make the natural loss of legs almost an impossible occurrence.

Perfectly protected against such natural enemies as bite off legs, there has certainly been nothing of that sharp necessity, during all the life of countless successive generations of silkworms, which is supposed to be the basis for maintaining the advantageous capacity for regeneration. There has been a clear field for panmixia. But the regenerative capacity still exists in effective degree. The silkworm offers little aid and comfort to those who would explain regeneration wholly as a phenomenon fostered and maintained by natural selection on a basis of utility.





# INFLUENCE OF THE PRIMARY REPRODUCTIVE ORGANS ON THE SECONDARY SEXUAL CHARACTERS.

BY

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This paper records the results of certain experiments on the silkworm moth, *Bombyx mori*, testing (a) the possibility of the regeneration of such important internal organs as the reproductive glands, and (b) the influence on the secondary sexual characters of the moth which the extirpation of the reproductive organs on one or both sides in larvæ of various ages might effect.

The first question is a general one in regeneration, touching particularly the relation of natural selection as a causative agent to regeneration: external organs liable to natural mutilation might be perhaps advantageously regenerative, but internal organs not liable to mutilation could not, if regenerative, have this capacity of theirs explicable as a result of selection. The second question concerns the immediate stimulus which leads to the development in any individual of the secondary sexual characters (ornamental tufts of hair, feathers or scales, defensive, offensive or attractive processes, markings, etc., the peculiar clasping organs, etc., composing the external genitalia). Certain biologists hold that the stimulus for these secondary sexual characters in any individual depends directly upon the presence of the primary reproductive organs (ovaries or testes).

J. W. Tutt<sup>1</sup> discusses the latter question, quoting the entomologist Wood, as follows: "The functions, then, of the reproductive glands are twofold: on the one hand they supply germ matter that resides within them with the means of developing and

<sup>1</sup>Entomologists Record, 1900, Vol. XII, pp. 199-202.

multiplying, and on the other hand they modify and even originate those parts of the soma which are lumped together under the name of secondary sexual characters." Again, Wood says, "Much of the variation that we find in male appendages (of *Lithocolletids*, small moths) is of a neutral character, neither useful nor hurtful to them as clasping organs. All this amazing fertility of shape is dependent in some way upon the presence of the reproductive glands (testes) for it can scarcely be doubted that they could be removed at a sufficiently early date in the life of the larva, the transformation of the last larval segment into the armature of the imago would not occur, much as the emasculation of the deer prevents the development of its horns."

These positive declarations of Wood are based of course on no evidence except that suggested in the last phrase of the quotation, but are nevertheless of interest as an expression of a problem which has important bearings. To biologists of the epigenetic school holding that the successive phenomena of development find their causative stimuli in the phenomena that have preceded them, the theory that the secondary sexual characters of insects, so numerous and so conspicuous in their divergence in the two sexes, should find a sufficient and immediate stimulus in the pre-development of the primary organs, would be a reasonable one.

The statement of my experiments with the silkworm moth, *Bombyx mori*, to follow, will show that for one species of insect at least this theory can be directly tested. It should be noted that those parts of the adult insect (having a complete metamorphosis, which is the case with the silkworm moth) which show the secondary sexual characters, as antennæ, wings, legs, external genitalia, etc., develop not from corresponding parts in the larva (in most cases no corresponding parts even existing in the larva) but from histoblasts or so-called imaginal discs, which are derived during larval life by the invagination at specific points of the larval skin layer (hypoderm), and which begin to reach in their development the character of the definitive imaginal (adult) parts only in very late larval (pre-pupal) and in pupal life. The primary reproductive organs are on the contrary developed early in larval life (or

in the egg) and in the case of the silkworm moth, at least, become obviously differentiated as ovaries or testes long before the secondary characters have made any beginning at all at differentiation. If, therefore, the primary organs (ovaries, testes) of a silkworm could be removed at any time before the last larval moulting, any direct stimulus or influence of these organs on the later developing secondary characters, would be prevented.

After the experiments described below had been completed my attention was called to the previous experiments of the same nature by Dr. J. Th. Oudemans on the gypsy moth, *Ocneria dispar*. Dr. Oudemans (in 1895 and 1896) removed variously the right or left or both reproductive organs of larvæ of *Ocneria* and noted that in the moths developed from these larvæ no modification whatever of the secondary sexual characters appeared. Of practically all the results obtained by Dr. Oudemans<sup>1</sup> with *Ocneria dispar* my own observations on *Bombyx mori* are wholly confirmatory. In the light of the fact that the removal in early life of the reproductive organs of vertebrates affects in marked degree the development of the secondary sexual characteristics, the absence of any such effect in insects (at least in the only ones yet experimented on) introduces to biologists an interesting problem.

Herbst<sup>2</sup> suggests that as the primary reproductive organs are differentiated (as to sexual character) early in larval life, their influence on the presumably already developing secondary sexual characters has been exercised before their artificial extirpation. As a matter of fact although the histoblasts of those imaginal organs which show secondary sexual characters as wings, antennæ, etc., may in some cases be distinguished from the rest of the larval derm in early larval life, no approximation to actual wings or antennæ exist until much later, and no distinction between male and female wings or antennæ, etc., until a very late stage in the larval life. In some cases, indeed, the histoblasts are not apparent until a considerable part of the larval life has been passed, while, as I have definitely determined for the silk worm, *Bombyx mori*,

<sup>1</sup>Zool. Jahrb. Abt. Syst., 1898, Vol. XII, pp. 71-88.

<sup>2</sup>Formative Reize, 1901, p. 79.



the sex of the larva can be distinguished by the character of its developing primary reproductive organs, at least immediately after the first larval moulting, if not, indeed, immediately after hatching.

The character of my experiments is as follows: a number of small lots of larvæ were taken, variously after the second, third and fourth moultings, and by means of a hot needle the right or left reproductive organ (ovary or testis) or both organs, were burned out. The developing reproductive organs in the larvæ lie just underneath the body wall of the fifth abdominal segment, and their position is indicated by a pair of small grayish tubercles. The slight wounds made by searing soon closed and the larvæ were reared to moths. Many larvæ died, especially among the earlier lots treated, some of these deaths being undoubtedly due to the mutilation. Sometimes the needle penetrated too deeply, searing the wall of the alimentary canal; sometimes the dorsal vessel (heart) lying very near the reproductive glands was injured; sometimes the destroying of the gland itself seemed sufficient to produce death. On the other hand in a very few cases I did not succeed in destroying the gland, or succeeded in searing away but part of it. But the results as a whole show very definite uniformity of result and afford positive conclusions.

Eight lots of larvæ of from five to twelve each were used, two lots being taken just after the second moulting, three after the third, and three after the fourth. In three of the lots the right reproductive gland was seared in each larva, in three the left gland was seared and in two the glands on both sides were seared. A few larvæ when full grown were dissected to note the condition of the reproductive glands. From the others eighteen moths were obtained. These moths were killed and dissected; in sixteen moths, including males and females, there was no trace of those reproductive glands, left or right or both, which had been seared in the larvæ; in one male moth both testes were perfect (undoubtedly a "miss" in the searing), in another male one testis was perfect while the other (seared in the larva) was very small and incomplete (either a case of regeneration or more probably a case of partial development of an incompletely destroyed larval



gland). There was no case of the absence or modification of the secondary sexual characters in any of these eighteen moths. All males had both antennæ of the usual male type although the testis of one side or the other was wholly wanting, or even both were absent.

The experiments prove that for *Bombyx mori* (*a*) there is no regeneration of mutilated or destroyed developing reproductive glands even though the glands be destroyed or mutilated as early as just after the second larval moulting, and (*b*) that the destruction of the primary reproductive organs (ovaries or testes) before the secondary sexual characters are developed has no effect on the normal course of development of these characteristics.



# STUDIES IN THE EVOLUTION OF PECTEN.<sup>1</sup>

## IV. RAY VARIABILITY IN PECTEN VARIUS.

BY

C. B. DAVENPORT AND MARIAN E. HUBBARD.

### I. STATEMENT OF THE PROBLEM.

Previous studies on the variability in the number of the rays of *Pecten* have demonstrated that this number is, during development, quite removed from any effect of external environment, excepting only mutilation. The rays form, consequently, excellent material for the study of variability inherent in the germ-plasm. Three species of *Pecten* have been previously quantitatively studied, all belonging to one group, sometimes known as the subgenus *Aequipecten* (cf. Verrill, '99), characterized by large, round, and nearly equal valves with fewer than 25 rays. It seemed desirable to compare with these the variability of species belonging to another group of the genus characterized by dorso-ventrally elongated and skew valves and by more than 25 rays. For this purpose *Pecten varius* seems admirably adapted.

In comparing the variability of the rays of a species of the *varius* type with that of species of the subgenus *Aequipecten* two points of interest will be to learn whether the skewness remains slight as in *Aequipecten* and, secondly, whether variability runs parallel with the mean, increasing and diminishing with it.

### II. MATERIAL AND METHOD.

The shells of *Pecten varius* used in this study were collected between November 12, 1902, and February, 1903, from the lagoon (Etang de Thau) between Cette and the mainland of

<sup>1</sup>Titles of Nos. I to III of this series are given in the literature list as Davenport 1900<sup>e</sup>, 1903<sup>c</sup> and 1903<sup>d</sup>, respectively.

the Mediterranean coast of France. Dr. Louis Calvet, in charge of Marine Zoölogical Station of the Université de Montpellier at Cette, kindly arranged for the collecting of these shells and the shipping of them to America.<sup>1</sup>

The counting of the rays of the shells was done by the junior author after a preliminary joint count had been made on a number of identical shells to decide upon the standards to be adopted in counting. Since the interior grooves corresponding to the exterior ridges are not always clearly marked the external ridges were counted. Now, the counting of rays in *Aequipecten* was made on the interior grooves, and since there are always a few<sup>2</sup> lateral ridges not represented by interior grooves (and so not enumerated) the averages cannot be too closely contrasted with those of *Aequipecten*, although decidedly greater. A slight difficulty is introduced by the fact that at the lateral ends of the series, rays are sometimes imperfect or ambiguous; the rule adopted was this: all rays, however faint, that reach the margin of the shell were counted in provided they extended centrad beyond the distal limit of the "ears." The shells having been numbered, the count for each individual shell was recorded and later each determination was checked by a recount.

The data were seriated and subjected to the usual quantitative analysis. All calculations were checked by being made independently by each of us.

### III. RESULTS.

The most striking difference between the system of rays of *P. varius* and that of *P. opercularis-irradians* type is found in the greater number of irregularities in the rays of the former. Such irregularities are relatively rare in *P. irradians* (Davenport, 1900<sup>6</sup>, p. 879); commoner in *P. opercularis* (1903<sup>d</sup>, p. 138); but are found in a large percentage of the shells of *P. varius*. About 12½ per cent have rays double, or one ray interpolated, or one, as

<sup>1</sup> I take this opportunity to acknowledge the generosity with which the privileges of the beautiful Station at Cette were accorded Mrs. Davenport and myself in November, 1902, by Professor Sabatier, the Director of the Station, and Dr. Calvet.  
C. B. Davenport.

<sup>2</sup> One to five.



it were, budded from the side of another. About 8 per cent of the shells show marked breaks in the continuity of the surface, due to the edge of the valves in the 15-20 mm. shell having met at a less acute angle than earlier and later. Out of 500 shells 60 showed interpolated or double rays.

We found:

8 extra rays	1 time.
4 " "	1 "
2 " "	14 times.
1 " "	44 "

In the cases with two extra rays, one ray was long enough to count in accordance with our rule in 3 cases; both rays were so long in 6 cases; and neither was counted in 5 cases. Of the cases with one extra ray, the ray was counted in 23 cases.

TABLE OF RAY-FREQUENCIES, THEIR DEVIATIONS AND THEIR MOMENTS.

Classes	$f(v-v_0)$	$f(v-v_0)$	$f(v-v_0)^2$	$f(v-v_0)^3$	$f(v-v_0)^4$
26	3 -4	- 12	48	-192	768
27	14 -3	- 42	126	-378	1134
28	55 -2	-110	220	-440	880
29	88 -1	- 88	88	- 88	88
30	122 0	-252		-1098	
31	97 1	97	97	97	97
32	75 2	150	300	600	1200
33	35 3	105	315	945	2835
34	7 4	28	112	448	1792
35	3 5	15	75	375	1875
36	1 6	6	36	216	1296
	500	401	1417	2681	11965
		-252		-1098	
		149		1583	
$\nu_1 = .298$	$\nu_2 = 2.834$	$\nu_3 = 3.166$	$\nu_4 = 23.930$		
	$\mu_2 = 2.7452$	$\mu_3 = 0.6853$	$\mu_4 = 21.6424$		

The foregoing analysis of the distribution of frequencies by moments shows us that the distribution apparently falls into

Pearson's Type I, with slight positive skewness. But the distribution is so nearly normal that it may be taken as essentially so.

The following table gives a comparative view of the constants for the different species of *Pecten* that have been quantitatively studied.

TABLE OF CONSTANTS FOR NUMBER OF RAYS IN THE RIGHT (LOWER) VALVE.

	n	A	$\sigma$	V	$\alpha$
<i>Pecten irradians</i> :					
Cold Spring Harbor...	1046	17.353 $\pm$ .118	0.876 $\pm$ .013	5.049% $\pm$ .074	+0.023
Cutchogue, L. I. ....	281	16.534 $\pm$ .034	0.852 $\pm$ .024	5.15 % $\pm$ .15	+0.025
<i>Pecten gibbus</i> :					
Dunedin (Tampa), Fla. ....	502	20.512 $\pm$ .030	0.991 $\pm$ .021	4.83 % $\pm$ .10	+0.104
<i>Pecten ventricosus</i> :					
San Diego, Cal. ....	471	19.459 $\pm$ .087	0.885 $\pm$ .019	4.55 % $\pm$ .10	+0.015
<i>Pecten opercularis</i> :					
Eddystone Light ....	536	17.478 $\pm$ .029	1.000 $\pm$ .020	5.72 % $\pm$ .12	+ .080
Irish Sea ....	614	18.101 $\pm$ .029	1.074 $\pm$ .021	5.931% $\pm$ .114	+ .087
Firth of Forth ....	508	17.673 $\pm$ .027	1.117 $\pm$ .019	6.32 % $\pm$ .11	+ .0069
<i>Pecten varius</i> , Cette ..	500	30.298 $\pm$ .050	1.657 $\pm$ .035	5.56 % $\pm$ .11	+ .067

#### IV. CONCLUSIONS.

##### I. Skewness.

Comparing the skewness of the frequency distribution of *varius* with that of other species we find that, although the species marks a departure from the more usual number of rays yet the skewness of the distribution remains, like that of the other species, small. The skewness in the *Pecten* ray distributions, so far as studied, rises only exceptionally to 0.1, although in other data this amount of skewness is by no means uncommon.

Now, large skewness is frequently, if not usually, associated with recent or progressing evolutionary change. Thus in long and in short-winged chinch bugs, which probably represent a recent division of a monomorphic group, the skewness in the distribution of wing-length is  $-.43$  and  $+.44$ , respectively; in the short-horned race of rhinoceros beetles it is  $-.48$ . In the number of lips of the newly arisen species of medusa, *P. pentata*, it is  $-.55$  (Mayer, 1901). In the shell-index of the newly introduced

shore snail, *Littorina littorea*, of Newport, R. I., it is  $-.25$ . In the ray-number of a selected 12-rayed race of *Chrysanthemum* it is  $-.19$ , and so on. On the other hand only in some fossil *Pecten irradians* of Virginia ( $\alpha=.22$ ) do we find a skewness much exceeding  $0.1$ . We may conclude, consequently, that, so far as the number of their rays is concerned, modern *Pectens* of the *P. irradians* and *P. varius* types are not undergoing rapid evolution.

## 2. Variability.

The name *varius* implies an unusual variability in the species we have studied. This variability is especially striking in the color of the shell which varies from a straw color to a red and almost to a black. We have seen, also, that there are frequent abnormalities in the rays. Also, the standard deviation of the ray-number, as the preceding table shows, is much higher than in any of the *P. irradians* group, being about twice as great as in the *irradians* of Long Island and 50 per cent greater than in the *opercularis* from the Firth of Forth. The ray-number, then, seems also to justify the name *varius*.

But why is this species so variable although (as we may judge from the small index of skewness) undergoing no rapid phylogenetic change? It appears to us that the high variability in the number of rays depends in some way upon the great average number of rays. *Pecten varius*, like its close allies, is characterized by its great number of rays, a number hardly exceeded, so far as I know, in the entire vast genus. The exceptional variability is, indeed, closely proportional to the exceptional number of rays, so that if we divide the index of variability by the average number of rays we get for the resulting coefficient of variability of the form-unit a number, 5.56 per cent, which is no longer extreme but lies in the middle of the series of coefficients found in the *Aequipecten* group, running from 4.55 per cent to 6.32 per cent. Thus, while the index of variability is exceptional the coefficient of variability is not at all so.

The question arises, Why in a shell with many rays should the index of variability of the number of rays be greater than in a shell

with few rays? After some experience with numerically varying qualities one comes to expect such a relation. Moreover, the fact seems not difficult to explain. One of the factors of variability is the very complexity of the developmental process—the improbability that, in development, all component events will occur in exactly the same degree, number and order. The fewer the elementary events, the less the chance for a wide deviation from the average condition; the more numerous the events, the greater the number of elements capable of deviating, the greater the probability of a large deviation—indeed, the greater the probable deviation. This law is illustrated in the cleavage of eggs. In early cleavage stages cells of the same generation may cleave simultaneously; but in later cleavage stages even cells in symmetrically placed pairs may come to cleave at slightly different times. For the time of cleavage depends on many elements. When these have become very numerous, as in later cleavage stages, the chances that the accelerating and the retarding influences shall be exactly equal in the history of the two cells become smaller. It appears, then, as a general rule of variability that the fewer the variable parts the less the variability.

The whole matter of the proper measure of variability is a complex one. Some years ago Verschaffelt ('94), Pearson ('96) and one of us in a note to Brewster's ('97) paper independently proposed that the coefficient of variability be employed; that is, the ratio of the index of variability (square root of mean square deviation) to the average. Weldon ('97) for a time opposed the use of the coefficient in place of the index and Duncker ('00) discovered a case that he believed to demonstrate the meaninglessness of the coefficient. The two shrimps *Palaeonetes vulgaris* and *P. varians* are nearly related. Yet the former has, on the average, 8.3 spines on the rostrum and the latter 4.3 spines. Now Duncker assumes that the variability of a race will be much more constant than its mean. As a matter of fact the index of variability of the dorsal spines is .81 and .86 in the two species, respectively. The coefficients are 9.83 per cent and 20.00 per cent, respectively. The coefficients must be, Duncker concludes, meaningless. But since the assumption of the greater conservativeness of the index



over the mean has never been proved and is not justified the argument falls to the ground.

The advantage of the coefficient is that it is expressed in a universal unit, percentage; whereas the index is a concrete number expressible in the greatest possible diversity of units. For measured, graduated, variates the coefficient gives the only possible means of comparing two qualities measured by different standards. We cannot compare the variability of a race of people in stature and in weight by comparing their indices of variability; for what relation has a centimeter to a kilogram? We must therefore use the coefficients of variability for comparison or nothing. In the case of integral variates, on the other hand, there is a basis of comparison even though the integers are concrete. Four petals and two spines have at least this in common that they are four unit objects and two unit objects, respectively, and the latter is half of the former. In integral variates we can, consequently, compare the indices of variability.

Now, as to the preference between the index and the coefficient of variability a proper doubt may exist. In comparing variabilities should the different planes from which variability must be measured be frankly conceded and, as in altitude determinations, consideration be given only to the end result (the index of variability) without inquiry as to its favoring factors; or should one attempt, as in charting isobars from various barometric readings, to reduce to a common level?

It seems to us that both measures—both methods—are useful, but for different ends. To illustrate, the altitude of a mountain peak above sea-level is significant when we wish to know the effect of altitude on barometric pressure or upon temperature; the altitude of a peak above the plain at its base is significant when we wish to measure the mountain-forming movements. So in variation studies, the absolute amount of variability (the index) in the numbers of a numerically repeated organ is important as indicating the range of opportunity afforded for evolution in different directions. A *Pecten* with only 5 rays with an index of variability of  $\frac{1}{3}$  ray offers less opportunity for the production of a new race with a new number of rays than does a *Pecten* with

30 rays and an index of 2. The higher index indicates the higher potentiality in evolution.

On the other hand the coefficient of variability in integral variates marks rather the inherent capacity to vary—not of the system as a whole—but of each of its numerically repeated parts. It disentangles, as it were, the variability due to complexity or manifoldness of structure and the inherent variability of parts. Comparing the two we may say the index is concerned only with the end-result and makes no allowance for the complexity of the contributory causes; it measures, in a way, the complexity combined with the variability of the germ-plasm. The coefficient, on the contrary, more nearly measures the relative variability of the germ-plasm alone.

The idea of the difference between complexity combined with variability of parts and variability alone may be assisted by an illustration. Imagine two projected railway lines of which the profiles are to be obtained by leveling. The one is very short, the other long. In leveling the shorter course, even though the level has to be set up only once, there are certain errors. These will be largely in the instrument itself due to its imperfect adjustment, or they may be due to errors in the graduation of the rod. These may be called the constant errors; they correspond to the inherent variability of the germ-plasm. In the longer course, where the level has to be set up many times, a different sort of error has to be taken into account. This is the sum of errors due to imperfect reading of the rod on the turning points, due to irregular heating of the sides of the telescope, due to diffraction of light by the atmosphere. This second sort of error is mostly outside of the instrument itself, and it tends to increase with the length of the course, and to a certain extent with its irregularity. Now, if two equally accurate leveling parties were getting the profile between two pairs of points of which the elevations are known, the distance of the one course being short and the other long, we should expect the absolute error at the end of the long course to be greater than at the end of the short course. In comparing the accuracy of the two parties we should feel justified in dividing the absolute error made by each by the length of the line traversed—expressing the

error as a percentage of that length. Nevertheless, the size of the absolute error is of great moment in such a survey. For an error in levels of one meter at the end of a 100 kilometer course is practically much more important than an error of a centimeter at the end of a single kilometer course. Exactly, then, as absolute and proportional errors have both their importance in engineering, so neither the index of variability nor the coefficient can be neglected in biology. Each has its peculiar significance.

#### V. SUMMARY.

*Pecten varius* of Cotte has a large number of rays as compared with representatives of other subgenera of *Pecten*. The frequency distribution has a small skewness as in other subgenera indicating that the species is probably not evolving rapidly. The variability of the ray frequency is large when measured by the index, but the same as in other species with fewer average rays when measured by the coefficient of variability. Both index and coefficient give important insight, each of its own kind, as to the degree and nature of the variability.

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